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<b>(21) International Application Number:</b> PCT/US97/22623 <b>(22) International Filing Date:</b> 8 December 1997 (08.12.97)  <b>(30) Priority Data:</b> 60/056,916 6 December 1996 (06.12.96) US Not furnished 10 October 1997 (10.10.97) US  <b>(71) Applicant (for all designated States except US):</b> DIVERSA CORPORATION [US/US]; 10665 Sorrento Valley Road, San Diego, CA 92121 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> BYLINA, Edward, J. [US/US]; Apartment A-1, West Court, Andalusia, PA 19020 (US). SWANSON, Ronald, V. [US/US]; Apartment A, 309 No. Lemon Street, Media, PA 19063 (US). MATHUR, Eric, J. [US/US]; 2654 Galicia Way, Carlsbad, CA 92009 (US). LAM, David, E. [US/US]; 1518 West 249th Street, Harbor City, CA 90710 (US).  <b>(74) Agent:</b> HAILE, Lisa, A.; Fish & Richardson P.C., Suite 1400, 4225 Executive Square, La Jolla, CA 92037 (US).		<b>(81) Designated States:</b> AU, CA, IL, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> GLYCOSIDASE ENZYMES  <b>(57) Abstract</b>  Thermostable glycosidase enzymes derived from various Thermococcus, Staphylothermus and Pyrococcus organisms is disclosed. The enzymes are produced from native or recombinant host cells and can be utilized in the food processing industry, pharmaceutical industry and in the textile industry, detergent industry and in the baking industry.		

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## GLYCOSIDASE ENZYMES

## BACKGROUND OF THE INVENTION

1. *Field of the Inventions*

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production and isolation of such polynucleotides and polypeptides. More particularly, the polynucleotides and polypeptides of the present invention has been putatively identified as glucosidases,  $\alpha$ -galactosidases,  $\beta$ -galactosidases,  $\beta$ -mannosidases,  $\beta$ -mannanases, endoglucanases, and pullulanases.

2. *Description of Related Art*

The glycosidic bond of  $\beta$ -galactosides can be cleaved by different classes of enzymes: (i) phospho- $\beta$ -galactosidases (EC3.2.1.85) are specific for a phosphorylated substrate generated via phosphoenolpyruvate phosphotransferase system (PTS)-dependent uptake; (ii) typical  $\beta$ -galactosidases (EC 3.2.1.23), represented by the *Escherichia coli* LacZ enzyme, which are relatively specific for  $\beta$ -galactosides; and (iii)  $\beta$ -glucosidases (EC 3.2.1.21) such as the enzymes of *Agrobacterium faecalis*, *Clostridium thermocellum*, *Pyrococcus furiosus* or *Sulfolobus solfataricus* (Day, A.G. and Withers, S.G., (1986) Purification and characterization of a  $\beta$ -glucosidase from *Alcaligenes faecalis*. Can. J. Biochem. Cell. Biol. 64, 914-922; Kengen, S.W.M., et al. (1993) Eur. J. Biochem., 213, 305-312; Ait, N., Cruezet, N. and Cattaneo, J. (1982) Properties of  $\beta$ -glucosidase purified from *Clostridium thermocellum*. J. Gen. Microbiol. 128, 569-577; Grogan, D.W. (1991) Evidence that  $\beta$ -galactosidase of *Sulfolobus solfataricus* is only one of several activities of a thermostable  $\beta$ -D-glycosidase. Appl. Environ. Microbiol. 57, 1644-1649). Members of the latter group, although highly specific with respect to the  $\beta$ -anomeric configuration of the glycosidic linkage, often display a rather relaxed substrate specificity and hydrolyze  $\beta$ -glucosides as well as  $\beta$ -fucosides and  $\beta$ -galactosides.

Generally,  $\alpha$ -galactosidases are enzymes that catalyze the hydrolysis of galactose groups on a polysaccharide backbone or hydrolyze the cleavage of di- or oligosaccharides comprising galactose.

Generally,  $\beta$ -mannanases are enzymes that catalyze the hydrolysis of mannose groups internally on a polysaccharide backbone or hydrolyze the cleavage of di- or oligosaccharides comprising mannose groups.  $\beta$ -mannosidases hydrolyze non-reducing, terminal mannose residues on a mannose-containing polysaccharide and the cleavage of di- or oligosaccharides comprising mannose groups.

Guar gum is a branched galactomannan polysaccharide composed of  $\beta$ -1,4 linked mannose backbone with  $\alpha$ -1,6 linked galactose side chains. The enzymes required for the degradation of guar are  $\beta$ -mannanase,  $\beta$ -mannosidase and  $\alpha$ -galactosidase.  $\beta$ -mannanase hydrolyses the mannose backbone internally and  $\beta$ -mannosidase hydrolyses non-reducing, terminal mannose residues.  $\alpha$ -galactosidase hydrolyses  $\alpha$ -linked galactose groups.

Galactomannan polysaccharides and the enzymes that degrade them have a variety of applications. Guar is commonly used as a thickening agent in food and is utilized in hydraulic fracturing in oil and gas recovery. Consequently, galactomannanases are industrially relevant for the degradation and modification of guar. Furthermore, a need exists for thermostable galactomannanases that are active in extreme conditions associated with drilling and well stimulation.

There are other applications for these enzymes in various industries, such as in the beet sugar industry. 20-30% of the domestic U.S. sucrose consumption is sucrose from sugar beets. Raw beet sugar can contain a small amount of raffinose when the sugar beets are stored before processing and rotting begins to set in. Raffinose inhibits the crystallization of sucrose and also constitutes a hidden quantity of sucrose. Thus, there is merit to eliminating raffinose from raw beet sugar.  $\alpha$ -Galactosidase has also been used as a digestive aid to break down raffinose, stachyose, and verbascose in such foods as beans and other gassy foods.

$\beta$ -galactosidases which are active and stable at high temperatures appear to be superior enzymes for the production of lactose-free dietary milk products (Chaplin, M.F.

and Bucke, C. (1990) In: Enzyme Technology, pp. 159-160, Cambridge University Press, Cambridge, UK). Also, several studies have demonstrated the applicability of  $\beta$ -galactosidases to the enzymatic synthesis of oligosaccharides via transglycosylation reactions (Nilsson, K.G.I. (1988) Enzymatic synthesis of oligosaccharides. Trends Biotechnol. 6, 156-264; Cote, G.L. and Tao, B.Y. (1990) Oligosaccharide synthesis by enzymatic transglycosylation. Glycoconjugate J. 7, 145-162). Despite the commercial potential, only a few  $\beta$ -galactosidases of thermophiles have been characterized so far. Two genes reported are  $\beta$ -galactoside-cleaving enzymes of the hyperthermophilic bacterium *Thermotoga maritima*, one of the most thermophilic-organotrophic eubacteria described to date (Huber, R., Langworthy, T.A., König, H., Thomm, M., Woese, C.R., Sleytr, U.B. and Stetter, K.O. (1986) *T. maritima* sp. nov. represents a new genus of unique extremely thermophilic eubacteria growing up to 90°C, Arch. Microbiol. 144, 324-333) one of the most thermophilic organotrophic eubacteria described to date. The gene products have been identified as a  $\beta$ -galactosidase and a  $\beta$ -glucosidase.

Pullulanase is well known as a debranching enzyme of pullulan and starch. The enzyme hydrolyzes  $\alpha$ -1,6-glucosidic linkages on these polymers. Starch degradation for the production of sweeteners (glucose or maltose) is a very important industrial application of this enzyme. The degradation of starch is developed in two stages. The first stage involves the liquefaction of the substrate with  $\alpha$ -amylase, and the second stage, or saccharification stage, is performed by  $\beta$ -amylase with pullulanase added as a debranching enzyme, to obtain better yields.

Endoglucanases can be used in a variety of industrial applications. For instance, the endoglucanases of the present invention can hydrolyze the internal  $\beta$ -1,4-glycosidic bonds in cellulose, which may be used for the conversion of plant biomass into fuels and chemicals. Endoglucanases also have applications in detergent formulations, the textile industry, in animal feed, in waste treatment, and in the fruit juice and brewing industry for the clarification and extraction of juices.

### **Brief Description of the Drawings**

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figures 1a-b are the full-length DNA and corresponding deduced amino acid sequence of M11TL of the present invention. Sequencing was performed using a 378 automated DNA sequencer for all sequences of the present invention (Applied Biosystems, Inc.).

Figure 2 is an illustration of the full-length DNA and corresponding deduced amino acid sequence of OC1/4V-33B/G.

Figure 3 is an illustration of the full-length DNA and corresponding deduced amino acid sequence of F1-12G.

Figures 4a-b are the full-length DNA and corresponding deduced amino acid sequence of 9N2-31B/G.

Figures 5a-b are the full-length DNA and corresponding deduced amino acid sequence of MSB8-6G.

Figure 6 is the full-length DNA and corresponding deduced amino acid sequence of AEDII12RA-18B/G.

Figures 7a-b are the full-length DNA and corresponding deduced amino acid sequence of GC74-22G.

Figures 8a-b are the full-length DNA and corresponding deduced amino acid sequence of VC1-7G1.

Figures 9a-c are the full-length DNA and corresponding deduced amino acid sequence of 37GP1.

Figures 10a-c are the full-length DNA and corresponding deduced amino acid sequence of 6GC2.

Figures 11a-d are the full-length DNA and corresponding deduced amino acid sequence of 6GP2.

Figures 12a-c are the full-length DNA and corresponding deduced amino acid sequence of 63GB1.

Figures 13a-b are the full-length DNA and corresponding deduced amino acid sequence of OC1/4V.

Figures 14a-e are the full-length DNA and corresponding deduced amino acid sequence of 6GP3.

5        Figures 15a-d are the full-length DNA and corresponding deduced amino acid sequence of *Thermotoga maritima* MSB8-6GP2.

Figures 16a-c are the full-length DNA and corresponding deduced amino acid sequence of *Thermotoga maritima* MSB8-6GB4.

10       Figures 17a-d are the full-length DNA and corresponding deduced amino acid sequence of *Banki gouldi* 37GP4.

Figures 18a-b are the full-length DNA and corresponding deduced amino acid sequence of *Pyrococcus furiosus* VC1-7EG1.

### SUMMARY OF THE INVENTION

15       In a preferred embodiment of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode mature enzymes having the deduced amino acid sequences of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64).

20       In another embodiment, the invention provides a method for producing a polypeptide including culturing host cells containing the polynucleotide of Figures 1-18 and expressing from the host cell a polypeptide encoded by the polynucleotide and isolating the polypeptide.

In another embodiment, the invention provides an enzyme selected from the group consisting of an enzyme having an amino acid sequence set forth in SEQ ID NOS: 15-28 or 61-64 and an enzyme which has at least 30 consecutive amino acid residue as an enzyme having an amino acid sequence set forth in SEQ ID NOS: 15-28 or 61-64.

25       In yet another embodiment, the invention provides a method for generating glucose from soluble cell oligosaccharides which includes contacting a sample containing oligosaccharides with an effective amount of an enzyme selected from the group of

enzymes having the amino acid sequence set forth in SEQ ID NOS: 15-28, 61-63 and 64 such that glucose is produced

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

### Definitions

"Monosaccharide", as used herein, refers to a single polyhydroxy aldehyde or ketone unit.

"Oligosaccharide", as used herein, consist of short chains of monosaccharide units joined together by covalent bonds. Of these, the most abundant are the disaccharides, which have two monosaccharide units.

"Polysaccharide", as used herein, consists of long chains having many monosaccharide units.

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

A coding sequence is "operably linked to" another coding sequence when RNA polymerase will transcribe the two coding sequences into a single mRNA, which is then translated into a single polypeptide having amino acids derived from both coding sequences. The coding sequences need not be contiguous to one another so long as the expressed sequences ultimately process to produce the desired protein.

"Recombinant" enzymes refer to enzymes produced by recombinant DNA techniques; *i.e.*, produced from cells transformed by an exogenous DNA construct encoding the desired enzyme. "Synthetic" enzymes are those prepared by chemical synthesis.

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular enzyme, is a DNA sequence which is transcribed and translated into an enzyme when placed under the control of appropriate regulatory sequences.



### Detailed Description of the Invention

The polynucleotides and polypeptides of the present invention have been identified as glucosidases,  $\alpha$ -galactosidases,  $\beta$ -galactosidases,  $\beta$ -mannosidases,  $\beta$ -mannanases, endoglucanases, and pullulanases as a result of their enzymatic activity.

5 In accordance with one aspect of the present invention, there are provided novel enzymes, as well as active fragments, analogs and derivatives thereof.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules encoding the enzymes of the present invention including mRNAs, cDNAs, genomic DNAs as well as active analogs and fragments of such enzymes.

10 In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing a nucleic acid sequence of the present invention, under conditions promoting expression of said enzymes and subsequent recovery of said enzymes.

15 In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such enzymes, or polynucleotides encoding such enzymes for hydrolyzing lactose to galactose and glucose for use in the food processing industry, the pharmaceutical industry, for example, to treat intolerance to lactose, as a diagnostic reporter molecule, in corn wet milling, in the fruit juice industry, in baking, in the textile industry and in the detergent industry.

20 In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such enzymes for hydrolyzing guar gum (a galactomannan polysaccharide) to remove non-reducing terminal mannose residues. Further polysaccharides such as galactomannan and the enzymes according to the invention that  
25 degrade them have a variety of applications. Guar gum is commonly used as a thickening agent in food and also is utilized in hydraulic fracturing in oil and gas recovery. Consequently, mannanases are industrially relevant for the degradation and modification of guar gums. Furthermore, a need exists for thermostable mannanases that are active in extreme conditions associated with drilling and well stimulation.

In accordance with yet a further aspect of the present invention, there are also provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to a nucleic acid sequence of the present invention.

In accordance with yet a further aspect of the present invention, there is provided  
5 a process for utilizing such enzymes, or polynucleotides encoding such enzymes, for *in vitro* purposes related to scientific research, for example, to generate probes for identifying similar sequences which might encode similar enzymes from other organisms by using certain regions, *i.e.*, conserved sequence regions, of the nucleotide sequence.

These and other aspects of the present invention should be apparent to those skilled  
10 in the art from the teachings herein.

The polynucleotides of this invention were originally recovered from genomic gene libraries derived from the following organisms:

M11TL is a new species of *Desulfurococcus* isolated from Diamond Pool in Yellowstone National Park. The organism grows optimally at 85-88°C, pH 7.0 in a low salt  
15 medium containing yeast extract, peptone, and gelatin as substrates with a N<sub>2</sub>/CO<sub>2</sub> gas phase.

OC1/4V is from the genus *Thermotoga*. The organism was isolated from Yellowstone National Park. It grows optimally at 75°C in a low salt medium with cellulose as a substrate and N<sub>2</sub> in gas phase.

20 *Pyrococcus furiosus* VC1 and (7EG1) is from the genus *Pyrococcus*. VC1 was isolated from Vulcano, Italy. It grows optimally at 100°C in a high salt medium (marine) containing elemental sulfur, yeast extract, peptone and starch as substrates and N<sub>2</sub> in gas phase.

25 *Staphylothermus marinus* F1 is from the genus *Staphylothermus*. F1 was isolated from Vulcano, Italy. It grows optimally at 85°C, pH 6.5 in high salt medium (marine) containing elemental sulfur and yeast extract as substrates and N<sub>2</sub> in gas phase.

*Thermococcus* 9N-2 is from the genus *Thermococcus* 9N-2 was isolated from diffuse vent fluid in the East Pacific Rise. It is a strict anaerobe that grows optimally at 87°C.

*Thermotoga maritima* MSB8 and MSB8 (Clone # 6GP2 and 6GB4) is from the genus *Thermotoga*, and was isolated from Vulcano, Italy. MSB8 grows optimally at 85°C, pH 6.5 in a high salt medium (marine) containing starch and yeast extract as substrates and N<sub>2</sub> in gas phase.

*Thermococcus alcaliphilus* AEDII12RA is from the genus *Thermococcus*. AEDII12RA grows optimally at 85°C, pH 9.5 in a high salt medium (marine) containing polysulfides and yeast extract as substrates and N<sub>2</sub> in gas phase.

*Thermococcus chitonophagus* GC74 is from the genus *Thermococcus*. GC74 grows optimally at 85°C, pH 6.0 in a high salt medium (marine) containing chitin, meat extract, elemental sulfur and yeast extract as substrates and N<sub>2</sub> in gas phase. AEPII 1a grows optimally at 85°C at pH 6.5 in marine medium under anaerobic conditions. It has many substrates. *Bankia gouldi* is from the genus *Bankia*.

Accordingly, the polynucleotides and enzymes encoded thereby are identified by the organism from which they were isolated, and are sometimes hereinafter referred to as "M11TL" (Figure 1 and SEQ ID NOS:1 and 15), "OC1/4V-33B/G" (Figure 2 and SEQ ID NOS:2 and 16), "F1-12G" (Figure 3 and SEQ ID NOS:3 and 17), "9N2-31B/G" (Figure 4 and SEQ ID NOS:4 and 18), "MSB8" (Figure 5 and SEQ ID NOS:5 and 19), "AEDII12RA-18B/G" (Figure 6 and SEQ ID NOS:6 and 20), "GC74-22G" (Figure 7 and SEQ ID NOS:7 and 21), "VC1-7G1" (Figure 8 and SEQ ID NOS:8 and 22), "37GP1" (Figure 9 and SEQ ID NOS: 9 and 23), "6GC2" (Figure 10 and SEQ ID NOS: 10 and 24), "6GP2" (Figure 11 and SEQ ID NOS:11 and 25), "AEPII 1a" (Figure 12 and SEQ ID NOS:12 and 26), "OC1/4V" (Figure 13 and SEQ ID NOS:13 and 27), and "6GP3" (Figure 14 and SEQ ID NOS:28), "MSB8-6GP2" (Figure 15 and SEQ ID NOS:57 and 61), "MSB8-6GB4"(Figure 16 and SEQ ID NOS:58 and 62), "VC1-7EG1"(Figure 17 and SEQ ID NOS:59 and 63), and 37GP4 (Figure 18 and SEQ ID NOS:60 and 64).

The polynucleotides and polypeptides of the present invention show identity at the nucleotide and protein level to known genes and proteins encoded thereby as shown in Table 1.

Table 1

Clone	Gene/Protein with Closest Homology	Protein Identity	Nucleic Acid Identity
M11TL-29G	<i>Sulfolobus sulfataricus</i> DSM 1616/P1, $\beta$ - galactosidase	51%	55%
OC1/4V-33B/G	<i>Caldocellum</i> saccharolyticum, $\beta$ - glucosidase	52%	57%
<i>Staphylothermus</i> <i>marinus</i> F1-12G	<i>Bacillus polymyxa</i> , $\beta$ - galactosidase	36%	48%
<i>Thermococcus</i> 9N2- 31B/G	<i>Sulfolobus sulfataricus</i> ATCC 49255/MT4, $\beta$ - galactosidase	51%	50%
<i>Thermotoga maritima</i> MSB8-6G	<i>Clostridium thermocellum</i> bglB	45%	53%
<i>Thermococcus</i> AEDII12RA-18B/G	<i>Bacillus polymyxa</i> , $\beta$ - galactosidase	34%	48%
<i>Thermococcus</i> <i>chitonophagus</i> GC74- 22G	<i>Sulfolobus sulfataricus</i> ATCC 49255/MT4, $\beta$ - galactosidase	46%	54%

<i>Pyrococcus furiosus</i> VC1-7G1	Sulfolobus sulfataricus/MT-4 $\beta$ - galactosidase	46.4%	52.5%
<i>Thermotoga maritima</i> $\alpha$ -galactosidase (6GC2)	Pediococcus pentosaceus $\alpha$ -galactosidase	49%	29%
<i>Thermotoga maritima</i> $\beta$ -mannanase (6GP2)	Aspergillus aculeatus mannanase	56%	37%
AEP11 1a $\beta$ - mannosidase (63GB1)	Sulfolobus solfataricus $\beta$ - galactosidase	78%	56%
OC1/4V endoglucanase (33GP1)	Clostridium thermocellum endo-1,4- $\beta$ -endoglucanase	65%	43%
<i>Thermotoga maritima</i> pullulanase (6GP3)	Caldocellum saccharolyticum $\alpha$ - destrom 6 glucanohydrolase	72	53
<i>Bankia gouldi</i> mix Endoglucanase (37GP1)	None available		

The polynucleotides and enzymes of the present invention show homology to each other as shown in Table 2.

Table 2

Clone	Gene/Protein with Closest Homology	Protein Identity	Nucleic Acid Identity
<i>Staphylothermus marinus</i> F1-12G	<i>Thermococcus</i> AEDII12RA-18B/G, $\beta$ -galactosidase, glucosidase	55%	57%
<i>Thermococcus</i> 9N2-31B/G	<i>Thermococcus chitonophagus</i> GC74-22G-glucosidase	74%	66%
<i>Pyrococcus furiosus</i> VC1-7G1	<i>Pyrococcus furiosus</i> VC1-7B/G $\beta$ -galactosidase	46.4%	54%

All the clones identified in Tables 1 and 2 encode polypeptides which have  $\alpha$ -glycosidase or  $\beta$ -glycosidase activity.

This invention, in addition to the isolated nucleic acid molecules encoding the enzymes of the present invention, also provide substantially similar sequences. Isolated nucleic acid sequences are substantially similar if: (i) they are capable of hybridizing under conditions hereinafter described, to the polynucleotides of SEQ ID NOS: 1-14 and 57-60; (ii) or they encode DNA sequences which are degenerate to the polynucleotides of SEQ ID NOS: 1-14 and 57-60. Degenerate DNA sequences encode the amino acid sequences of SEQ ID NOS: 15-28 and 61-64, but have variations in the nucleotide coding sequences. As used herein, substantially similar refers to the sequences having similar identity to the sequences of the instant invention. The nucleotide sequences that are substantially the same can be identified by hybridization or by sequence comparison. Enzyme sequences that are substantially the same can be identified by one or more of the following: proteolytic digestion, gel electrophoresis and/or microsequencing.

One means for isolating the nucleic acid molecules encoding the enzymes of the present invention is to probe a gene library with a natural or artificially designed probe using art recognized procedures (see, for example: Current Protocols in Molecular Biology,

Ausubel F.M. *et al.* (EDS.) Green Publishing Company Assoc. and John Wiley Interscience, New York, 1989, 1992). It is appreciated to one skilled in the art that the polynucleotides of SEQ ID NOS: 1-14 and 57-60 or fragments thereof (comprising at least 12 contiguous nucleotides), are particularly useful probes. Other particular useful probes for this purpose are hybridizable fragments to the sequences of SEQ ID NOS: 1-14 and 57-60 (*i.e.*, comprising at least 12 contiguous nucleotides).

With respect to nucleic acid sequences which hybridize to specific nucleic acid sequences disclosed herein, hybridization may be carried out under conditions of reduced stringency, medium stringency or even stringent conditions. As an example of oligonucleotide hybridization, a polymer membrane containing immobilized denatured nucleic acids is first prehybridized for 30 minutes at 45°C in a solution consisting of 0.9 M NaCl, 50 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.0, 5.0 mM Na<sub>2</sub>EDTA, 0.5% SDS, 10X Denhardt's, and 0.5 mg/ml polyriboadenylic acid. Approximately 2 X 10<sup>7</sup> cpm (specific activity 4-9 X 10<sup>6</sup> cpm/ug) of <sup>32</sup>P end-labeled oligonucleotide probe are then added to the solution. After 12-16 hours of incubation, the membrane is washed for 30 minutes at room temperature in 1X SET (150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na<sub>2</sub>EDTA) containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at Tm 10°C for the oligonucleotide probe. The membrane is then exposed to auto-radiographic film for detection of hybridization signals.

Stringent conditions means hybridization will occur only if there is at least 90% identity, preferably at least 95% identity and most preferably at least 97% identity between the sequences. Further, it is understood that a section of a 100 bps sequence that is 95 bps in length has 95% identity with the 1090 bps sequence from which it is obtained. See J. Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory (1989) which is hereby incorporated by reference in its entirety. Also, it is understood that a fragment of a 100 bps sequence that is 95 bps in length has 95% identity with the 100 bps sequence from which it is obtained.

As used herein, a first DNA (RNA) sequence is at least 70% and preferably at least 80% identical to another DNA (RNA) sequence if there is at least 70% and preferably at

least a 80% or 90% identity, respectively, between the bases of the first sequence and the bases of the another sequence, when properly aligned with each other, for example when aligned by BLASTN.

"Identity" as the term is used herein, refers to a polynucleotide sequence which comprises a percentage of the same bases as a reference polynucleotide (SEQ ID NOS:1-14 and 57-60). For example, a polynucleotide which is at least 90% identical to a reference polynucleotide, has polynucleotide bases which are identical in 90% of the bases which make up the reference polynucleotide and may have different bases in 10% of the bases which comprise that polynucleotide sequence.

The present invention relates polynucleotides which differ from the reference polynucleotide such that the changes are silent changes, for example the change do not alter the amino acid sequence encoded by the polynucleotide. The present invention also relates to nucleotide changes which result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference polynucleotide. In a preferred aspect of the invention these polypeptides retain the same biological action as the polypeptide encoded by the reference polynucleotide.

It is also appreciated that such probes can be and are preferably labeled with an analytically detectable reagent to facilitate identification of the probe. Useful reagents include but are not limited to radioactivity, fluorescent dyes or enzymes capable of catalyzing the formation of a detectable product. The probes are thus useful to isolate complementary copies of DNA from other sources or to screen such sources for related sequences.

The polynucleotides of this invention were recovered from genomic gene libraries from the organisms listed in Table 1. For example, gene libraries can be generated in the Lambda ZAP II cloning vector (Stratagene Cloning Systems). Mass excisions can be performed on these libraries to generate libraries in the pBluescript phagemid. Libraries are thus generated and excisions performed according to the protocols/methods hereinafter described.



The excision libraries are introduced into the *E. coli* strain BW14893 F<sup>'kan</sup>1A. Expression clones are then identified using a high temperature filter assay. Expression clones encoding several glucanases and several other glycosidases are identified and repurified. The polynucleotides, and enzymes encoded thereby, of the present invention, yield the activities as described above.

The coding sequences for the enzymes of the present invention were identified by screening the genomic DNAs prepared for the clones having glucosidase or galactosidase activity.

An example of such an assay is a high temperature filter assay wherein expression clones were identified by use of high temperature filter assays using buffer Z (see recipe below) containing 1 mg/ml of the substrate 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucopyranoside (XGLU) (Diagnostic Chemicals Limited or Sigma) after introducing an excision library into the *E. coli* strain BW14893 F<sup>'kan</sup>1A. Expression clones encoding XGLUases were identified and repurified from M11TL, OC1/4V, *Pyrococcus furiosus* VC1, *Staphylothermus marinus* F1, *Thermococcus* 9N-2, *Thermotoga maritima* MSB8, *Thermococcus alcaliphilus* AED112RA, and *Thermococcus chitonophagus* GC74.

Z-buffer: (referenced in Miller, J.H. (1992) A Short Course in Bacterial Genetics, p. 445.)

per liter:

$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$	16.1g
$\text{NaH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$	5.5g
KCl	0.75g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.246g
$\beta$ -mercaptoethanol	2.7ml
Adjust pH to 7.0	

#### High Temperature Filter Assay

- (1) The f factor f<sup>'kan</sup> (from *E. coli* strain CSH118)(1) was introduced into the pho-pnh-lac-strain BW14893(2). BW13893(2). The filamentous phage library was plated on the resulting strain, BW14893 F<sup>'kan</sup>. (Miller, J.H. (1992) A Short Course in

Bacterial Genetics; Lee, K.S., Metcalf, et al., (1992) Evidence for two phosphonate degradative pathways in *Enterobacter Aerogenes*, *J. Bacteriol.*, 174:2501-2510.

(2) After growth on 100 mm LB plates containing 100 µg/ml ampicillin, 80 µg/ml nethicillin and 1mM IPTG, colony lifts were performed using Millipore HATF membrane filters.

(3) The colonies transferred to the filters were lysed with chloroform vapor in 150 mm glass petri dishes.

(4) The filters were transferred to 100 mm glass petri dishes containing a piece of Whatman 3MM filter paper saturated with buffer.

(a) when testing for galactosidase activity (XGALase), 3MM paper was saturated with Z buffer containing 1 mg/ml XGAL (ChemBridge Corporation). After transferring filter bearing lysed colonies to the glass petri dish, placed dish in oven at 80-85°C.

(b) when testing for glucosidase (XGLUase), 3MM paper was saturated with Z buffer containing 1 mg/ml XGLU. After transferring filter bearing lysed colonies to the glass petri dish, placed dish in oven at 80-85°C.

(5) 'Positives' were observed as blue spots on the filter membranes. Used the following filter rescue technique to retrieve plasmid from lysed positive colony. Used pasteur pipette (or glass capillary tube) to core blue spots on the filter membrane. Placed the small filter disk in an Eppendorf tube containing 20 µl water. Incubated the Eppendorf tube at 75°C for 5 minutes followed by vortexing to elute plasmid DNA off filter. This DNA was transformed into electrocompetent *E. coli* cells DH10B for *Thermatoga maritima* MSB8-6G, *Staphylothermus marinus* F1-12G, *Thermococcus AEDII12RA-18B/G*, *Thermococcus chitonophagus* GC74-22G, M11T1 and OC1/4V. Electrocompetent BW14893 F<sup>kan</sup>1A *E. coli* were used for *Thermococcus* 9N2-31B/G, and *Pyrococcus furiosus* VC1-7G1. Repeated filter-lift assay on transformation plates to identify 'positives'. Return transformation plates to 37°C incubator after filter lift to regenerate colonies. Inoculate 3 ml LB liquid containing 100 µg/ml ampicillin with repurified positives and incubate at 37°C

overnight. Isolate plasmid DNA from these cultures and sequence plasmid insert. In some instances where the plates used for the initial colony lifts contained non-confluent colonies, a specific colony corresponding to a blue spot on the filter could be identified on a regenerated plate and repurified directly, instead of using the filter rescue technique.

Another example of such an assay is a variation of the high temperature filter assay wherein colony-laden filters are heat-killed at different temperatures (for example, 105°C for 20 minutes) to monitor thermostability. The 3MM paper is saturated with different buffers (i.e., 100 mM NaCl, 5 mM MgCl<sub>2</sub>, 100 mM Tris-Cl (pH 9.5)) to determine enzyme activity under different buffer conditions.

A  $\beta$ -glucosidase assay may also be employed, wherein Glcp $\beta$ Np is used as an artificial substrate (aryl- $\beta$ -glucosidase). The increase in absorbance at 405 nm as a result of p-nitrophenol (pNp) liberation was followed on a Hitachi U-1100 spectrophotometer, equipped with a thermostatted cuvette holder. The assays may be performed at 80°C or 90°C in closed 1-ml quartz cuvette. A standard reaction mixture contains 150 mM trisodium substrate, pH 5.0 (at 80°C), and 0.95 mM pNp derivative pNp = 0.561 mM<sup>-1</sup> cm<sup>-1</sup>). The reaction mixture is allowed to reach the desired temperature, after which the reaction is started by injecting an appropriate amount of enzyme (1.06 ml final volume).

1 U  $\beta$ -glucosidase activity is defined as that amount required to catalyze the formation of 1.0  $\mu$ mol pNp/min. D-cellobiose may also be used as a substrate.

An ONPG assay for  $\beta$ -galactosidase activity is described by Miller, J.H. (1992) A Short Course in Bacterial Genetics and Mill, J.H. (1992) Experiments in Molecular Genetics, the contents of which are hereby incorporated by reference in their entirety.

A quantitative fluorometric assay for  $\beta$ -galactosidase specific activity is described by : Youngman P., (1987) Plasmid Vectors for Recovering and Exploiting Tn917 Transpositions in Bacillus and other Gram-Positive Bacteria. In Plasmids: A Practical approach (ed. K. Hardy) pp 79-103. IRL Press, Oxford. A description of the procedure can be found in Miller (1992) p. 75-77, the contents of which are incorporated by reference herein in their entirety.

The polynucleotides of the present invention may be in the form of DNA which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequences which encodes the mature enzymes may be identical to the coding sequences shown in Figures 1-8 (SEQ ID NOS: 1-14 and 57-60) or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature enzymes as the DNA of Figures 1-18 (SEQ ID NOS: 1-14 and 57-60).

The polynucleotide which encodes for the mature enzyme of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) may include, but is not limited to: only the coding sequence for the mature enzyme; the coding sequence for the mature enzyme and additional coding sequence such as a leader sequence or a proprotein sequence; the coding sequence for the mature enzyme (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature enzyme.

Thus, the term "polynucleotide encoding an enzyme (protein)" encompasses a polynucleotide which includes only coding sequence for the enzyme as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the enzymes having the deduced amino acid sequences of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64). The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature enzymes as shown in Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the enzymes of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64). Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequences shown in Figures 1-18 (SEQ

ID NOS: 1-14 and 57-60). As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded enzyme.

5 Fragments of the full length gene of the present invention may be used as a hybridization probe for a cDNA or a genomic library to isolate the full length DNA and to isolate other DNAs which have a high sequence similarity to the gene or similar biological activity. Probes of this type preferably have at least 10, preferably at least 15, and even more preferably at least 30 bases and may contain, for example, at least 50 or more bases. The probe may also be used to identify a DNA clone corresponding to a full length  
10 transcript and a genomic clone or clones that contain the complete gene including regulatory and promotor regions, exons, and introns. An example of a screen comprises isolating the coding region of the gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of genomic DNA to  
15 determine which members of the library the probe hybridizes to.

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means  
20 hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode enzymes which either retain substantially the same biological function or activity as the mature enzyme encoded by the  
25 DNA of Figures 1-18 (SEQ ID NOS: 1-14 and 57-60).

Alternatively, the polynucleotide may have at least 15 bases, preferably at least 30 bases, and more preferably at least 50 bases which hybridize to any part of a polynucleotide of the present invention and which has an identity thereto, as hereinabove described, and which may or may not retain activity. For example, such polynucleotides may be employed

as probes for the polynucleotides of SEQ ID NOS: 1-14 and 57-60, for example, for recovery of the polynucleotide or as a diagnostic probe or as a PCR primer.

Thus, the present invention is directed to polynucleotides having at least a 70% identity, preferably at least 90% identity and more preferably at least a 95% identity to a polynucleotide which encodes the enzymes of SEQ ID NOS: 15-28 and 61-64 as well as fragments thereof, which fragments have at least 15 bases, preferably at least 30 bases and most preferably at least 50 bases, which fragments are at least 90% identical, preferably at least 95% identical and most preferably at least 97% identical under stringent conditions to any portion of a polynucleotide of the present invention.

The present invention further relates to enzymes which have the deduced amino acid sequences of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) as well as fragments, analogs and derivatives of such enzyme.

The terms "fragment," "derivative" and "analog" when referring to the enzymes of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) means enzymes which retain essentially the same biological function or activity as such enzymes. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature enzyme.

The enzymes of the present invention may be a recombinant enzyme, a natural enzyme or a synthetic enzyme, preferably a recombinant enzyme.

The fragment, derivative or analog of the enzymes of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature enzyme is fused with another compound, such as a compound to increase the half-life of the enzyme (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature enzyme, such as a leader or secretory sequence or a sequence which is employed for purification of the mature enzyme or a proprotein sequence. Such fragments, derivatives

and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The enzymes and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

5 The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or enzyme present in a living animal is not isolated, but the same polynucleotide or enzyme, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or  
10 such polynucleotides or enzymes could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The enzymes of the present invention include the enzymes of SEQ ID NOS: 15-28 and 61-64 (in particular the mature enzyme) as well as enzymes which have at least 70% similarity (preferably at least 70% identity) to the enzymes of SEQ ID NOS: 15-28 and 61-  
15 64 and more preferably at least 90% similarity (more preferably at least 90% identity) to the enzymes of SEQ ID NOS: 15-28 and 61-64 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the enzymes of SEQ ID NOS: 15-28 and 61-64 and also include portions of such enzymes with such portion of the enzyme generally containing at least 30 amino acids and more preferably at least 50 amino acids.

20 As known in the art "similarity" between two enzymes is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one enzyme to the sequence of a second enzyme.

A variant, i.e. a "fragment", "analog" or "derivative" polypeptide, and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions,  
25 deletions, fusions and truncations, which may be present in any combination.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala,

Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Most highly preferred are variants which retain the same biological function and activity as the reference polypeptide from which it varies.

Fragments or portions of the enzymes of the present invention may be employed for producing the corresponding full-length enzyme by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length enzymes. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of enzymes of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the present invention. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing enzymes by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing an enzyme. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.



The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

5 The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli lac or trp, the phage lambda P<sub>L</sub> promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also  
10 contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as  
15 tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial  
20 cells, such as E. coli, Streptomyces, Bacillus subtilis; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs  
25 comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and

promoters are known to those of skill in the art. and are commercially available. The following vectors are provided by way of example; Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pD10, psiX174, pBluescript II KS, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); Eukaryotic: pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P<sub>R</sub>, P<sub>L</sub> and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the enzymes of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory

Manual. Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the enzymes of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK),  $\alpha$ -factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated enzyme. Optionally, the heterologous sequence can encode a fusion enzyme including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from

commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell*, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The enzyme can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing

configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The enzymes of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the enzymes of the present invention may be glycosylated or may be non-glycosylated. Enzymes of the invention may or may not also include an initial methionine amino acid residue.

$\beta$ -galactosidase hydrolyzes lactose to galactose and glucose. Accordingly, the OC1/4V, 9N2-31B/G, AEDII12RA-18B/G and F1-12G enzymes may be employed in the food processing industry for the production of low lactose content milk and for the production of galactose or glucose from lactose contained in whey obtained in a large amount as a by-product in the production of cheese. Generally, it is desired that enzymes used in food processing, such as the aforementioned  $\beta$ -galactosidases, be stable at elevated temperatures to help prevent microbial contamination.

These enzymes may also be employed in the pharmaceutical industry. The enzymes are used to treat intolerance to lactose. In this case, a thermostable enzyme is desired, as well. Thermostable  $\beta$ -galactosidases also have uses in diagnostic applications, where they are employed as reporter molecules.

Glucosidases act on soluble cellooligosaccharides from the non-reducing end to give glucose as the sole product. Glucanases (endo- and exo-) act in the depolymerization of cellulose, generating more non-reducing ends (endo-glucanases, for instance, act on internal linkages yielding cellobiose, glucose and cellooligosaccharides as products).  $\beta$ -glucosidases are used in applications where glucose is the desired product. Accordingly, M11TL, F1-12G, GC74-22G, MSB8-6G, OC1/4V, VC1-7G1, 9N2-31B/G and AEDII12RA18B/G may be employed in a wide variety of industrial applications, including in corn wet milling for the separation of starch and gluten, in the fruit industry for clarification and equipment maintenance, in baking for viscosity reduction, in the textile

industry for the processing of blue jeans, and in the detergent industry as an additive. For these and other applications, thermostable enzymes are desirable.

Antibodies generated against the enzymes corresponding to a sequence of the present invention can be obtained by direct injection of the enzymes into an animal or by administering the enzymes to an animal, preferably a nonhuman. The antibody so obtained will then bind the enzymes itself. In this manner, even a sequence encoding only a fragment of the enzymes can be used to generate antibodies binding the whole native enzymes. Such antibodies can then be used to isolate the enzyme from cells expressing that enzyme.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, *Nature*, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic enzyme products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic enzyme products of this invention.

Antibodies generated against the enzyme of the present invention may be used in screening for similar enzymes from other organisms and samples. Such screening techniques are known in the art, for example, one such screening assay is described in "Methods for Measuring Cellulase Activities", *Methods in enzymology*, Vol 160, pp. 87-116, which is hereby incorporated by reference in its entirety.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described  
5 are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical  
10 purposes, typically 1  $\mu$ g of plasmid or DNA fragment is used with about 2 units of enzyme in about 20  $\mu$ l of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50  $\mu$ g of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are  
15 ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two  
20 complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two  
25 double stranded nucleic acid fragments (Maniatis, T., *et al.*, *Id.*, p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units of T4 DNA ligase ("ligase") per 0.5  $\mu$ g of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

### Example 1

#### Bacterial Expression and Purification of Glycosidase Enzymes

5 DNA encoding the enzymes of the present invention, SEQ ID NOS: 1-14 and 57-60 were initially amplified from a pBluescript vector containing the DNA by the PCR technique using the primers noted herein. The amplified sequences were then inserted into the respective PQE vector listed beneath the primer sequences, and the enzyme was expressed according to the protocols set forth herein. The 5' and 3' primer sequences for  
10 the respective genes are as follows:

##### *Thermococcus* AEDII12RA -18B/G

5' CCGAGAATTCATTAAAGAGGAGAGAAATTAAGTATGGTGAATGCTATGATTGTC 3' (SEQ ID NO:29)

3' CGGAAGATCTTCATAGCTCCGGAAGCCCATA 5' (SEQ ID NO:30)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Bgl  
15 II.

##### OC1/4V-33B/G

5' CCGAGAATTCATTAAAGAGGAGAGAAATTAAGTATGATAAGAAGGTCCGATTTTCC 3'

(SEQ ID NO:31)

3' CGGAAGATCTTTAAGATTTAGAAATTCCTT 5' (SEQ ID NO:32)

20 Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Bgl  
II.

##### *Thermococcus* 9N2 - 31B/G

5' CCGAGAATTCATTAAAGAGGAGAGAAATTAAGTATGCTACCAGAAGGCTTTCTC 3'

(SEQ ID NO:33)

25 3' CGGAGGTACCTCACCCAAGTCCGAATTCTC 5' (SEQ ID NO:34)

Vector: pQE30; and contains the following restriction enzyme sites 5' EcoRI and 3'  
KpnI.



*Staphylothermus marinus* F1 - 12G

5' CCGAGAATTCATTAAAGAGGAGAAATTAAGTTTCCTGATTAT 3'

(SEQ ID NO:35)

3' CGGAAGATCTTTATTCGAGGTTCTTAATCC 5' (SEQ ID NO:36)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Bgl

II.

*Thermococcus chitonophagus* GC74 - 22G

5' CCGAGAATTCATTCATTAAAGAGGAGAAATTAAGTTCCAGGAGAACTTTCTC 3'

(SEQ ID NO:37)

3' CGGAGGATCCCTACCCCTCCTAAGATCTC 5' (SEQ ID NO:38)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3'

BamHI.

## M11TL

5' AATAATCTAGAGCATGCAATCCCCAAAGACTTCATGATAG 3' (SEQ ID NO:39)

3' AATAAAAGCTTACTGGATCAGTGAAGATGCT 5' (SEQ ID NO:40)

Vector: pQE70; and contains the following restriction enzyme sites 5' SphI and 3' Hind

III.

*Thermotoga maritima* MSB8-6G

5' CCGACAATTGATTAAAGAGGAGAAATTAAGTTGGAAGGATCGATGAAATT 3' (SEQ ID NO:41)

3' CGGAGGTACCTCATGGTTTGAATCTCTTCTC 5' (SEQ ID NO:42)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3'

KpnI.

*Pyrococcus furiosus* VC1 - 7G1

5' CCGACAATTGATTAAAGAGGAGAAATTAAGTTCCCTGAAAAGTTCCTT 3' (SEQ ID NO:43)

3' CGGAGGTACCTCATCCCTCAGCAATTCCTC 5' (SEQ ID NO:44)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Kpn

I.

*Bankia gouldi* endoglucanase (37GP1)

5' AATAAGGATCCGTTAGCGACGCTCGC 3' (SEQ ID NO:45)

3' AATAAAAGCTTCCGGTTGTACAGCGGTAATAGGC 5' (SEQ ID NO:46)

Vector: pQE52; and contains the following restriction enzyme sites 5' Bam HI and 3'  
Hind III.

*Thermotoga maritima*  $\alpha$ -galactosidase (6GC2)

5' TTTATTGAATTCATTAAAGAGGAGAAATTAAGTATGATCTGTGTGGAAATATTCGGAAAG 3'  
(SEQ ID NO:47)

3' TCTATAAAGCTTTCATTCTCTCACCCTCTTCGTAGAAG 5' (SEQ ID NO:48)

Vector: pQET; and contains the following restriction enzyme sites 5' EcoRI and 3' Hind  
III.

*Thermotoga maritima*  $\beta$ -mannanase (6GP2)

5' TTTATTCAATTGATTAAAGAGGAGAAATTAAGTATGGGGATTGGTGGCGACGAC 3'  
(SEQ ID NO:49)

3' TTTATTAAGCTTATCTTTTCATATTCACATACCTCC 5' (SEQ ID NO:50)

Vector: pQEt; and contains the following restriction enzyme sites 5' Hind III and 3'  
EcoRI.

*AEPII 1a*  $\beta$ -mannanase (63GB1)

5' TTTATTGAATTCATTAAAGAGGAGAAATTAAGTATGCTACCAGAAGAGTTCCTATGGGGC 3'  
(SEQ ID NO:51)

3' TTTATTAAGCTTCTCATCAACGGCTATGGTCTTCATTTC 5' (SEQ ID NO:52)

Vector: pQEt; and contains the following restriction enzyme sites 5' Hind III and 3'  
EcoRI.

*OCI/4V* endoglucanase (33GP1)

5' AAAAAACAATTGAATTCATTAAAGAGGAGAAATTAAGTATGGTAGAAAGACACTTCAGATATGTTCTT  
3' (SEQ ID NO:53)

3' TTTTTCGGATCCAATTCTTCATTACTCTTTGCCTG 5' (SEQ ID NO:54)

Vector: pQEt; and contains the following restriction enzyme sites 5' BamHI and 3' EcoRI.

*Thermotoga maritima* pullulanase (6GP3)

5' TTTTGGGAATTCATTAAAGAGGAGAAATTAAGTATGGAACTGATCATAGAAGGTTAC 3'

(SEQ ID NO:55)

3' ATAAGAAGCTTTTCACTCTCTGTACAGAACGTACGC 5' (SEQ ID NO:56)

Vector: pQEt; and contains the following restriction enzyme sites 5' EcoRI and 3' Hind III.

The restriction enzyme sites indicated correspond to the restriction enzyme sites on the bacterial expression vector indicated for the respective gene (Qiagen, Inc. Chatsworth, CA). The pQE vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites.

The pQE vector was digested with the restriction enzymes indicated. The amplified sequences were ligated into the respective pQE vector and inserted in frame with the sequence encoding for the RBS. The ligation mixture was then used to transform the *E. coli* strain M15/pREP4 (Qiagen, Inc.) by electroporation. M15/pREP4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants were identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture was used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells were grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") was then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3 to 4 hours. Cells were then harvested by centrifugation.

The primer sequences set out above may also be employed to isolate the target gene from the deposited material by hybridization techniques described above.

### Example 2

#### Isolation of A Selected Clone From the Deposited genomic clones

5 A clone is isolated directly by screening the deposited material using the oligonucleotide primers set forth in Example 1 for the particular gene desired to be isolated. The specific oligonucleotides are synthesized using an Applied Biosystems DNA synthesizer. The oligonucleotides are labeled with  $^{32}\text{P}$ -ATP using T4 polynucleotide kinase and purified according to a standard protocol (Maniatis et al.,  
10 Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY, 1982). The deposited clones in the pBluescript vectors may be employed to transform bacterial hosts which are then plated on 1.5% agar plates to the density of 20,000-50,000 pfu/150 mm plate. These plates are screened using Nylon membranes according to the standard screening protocol (Stratagene, 1993). Specifically, the Nylon  
15 membrane with denatured and fixed DNA is prehybridized in 6 x SSC, 20 mM  $\text{NaH}_2\text{PO}_4$ , 0.4% SDS, 5 x Denhardt's 500  $\mu\text{g/ml}$  denatured, sonicated salmon sperm DNA; and 6 x SSC, 0.1% SDS. After one hour of prehybridization, the membrane is hybridized with hybridization buffer 6xSSC, 20 mM  $\text{NaH}_2\text{PO}_4$ , 0.4% SDS, 500  $\mu\text{g/ml}$  denatured, sonicated salmon sperm DNA with  $1 \times 10^6$  cpm/ml  $^{32}\text{P}$ -probe overnight at  
20 42°C. The membrane is washed at 45-50°C with washing buffer 6 x SSC, 0.1% SDS for 20-30 minutes dried and exposed to Kodak X-ray film overnight. Positive clones are isolated and purified by secondary and tertiary screening. The purified clone is sequenced to verify its identity to the primer sequence.

25 Once the clone is isolated, the two oligonucleotide primers corresponding to the gene of interest are used to amplify the gene from the deposited material. A polymerase chain reaction is carried out in 25  $\mu\text{l}$  of reaction mixture with 0.5  $\mu\text{g}$  of the DNA of the gene of interest. The reaction mixture is 1.5-5 mM  $\text{MgCl}_2$ , 0.01% (w/v) gelatin, 20  $\mu\text{M}$  each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq

polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with the Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the gene of interest by subcloning and sequencing the DNA product. The ends of the newly purified genes are nucleotide sequenced to identify full length sequences. Complete sequencing of full length genes is then performed by Exonuclease III digestion or primer walking.

### Example 3

#### Screening for Galactosidase Activity

Screening procedures for  $\alpha$ -galactosidase protein activity may be assayed for as follows:

Substrate plates were provided by a standard plating procedure. Dilute XL1-Blue MRF *E coli* host of (Stratagene Cloning Systems, La Jolla, CA) to O.D.<sub>600</sub> = 1.0 with NZY media. In 15 ml tubes, inoculate 200  $\mu$ l diluted host cells with phage. Mix gently and incubate tubes at 37 °C for 15 min. Add approximately 3.5 ml LB top agarose (0.7%) containing 1mM IPTG to each tube and pour onto all NYZ plate surface. Allow to cool and incubate at 37 °C overnight. The assay plates are obtained as substrate p-Nitrophenyl  $\alpha$ -galactosidase (Sigma) (200 mg/100 ml) (100 mM NaCl, 100 mM Potassium-Phosphate) 1% (w/v) agarose. The plaques are overlayed with nitrocellulose and incubated at 4 °C for 30 minutes whereupon the nitrocellulose is removed and overlayed onto the substrate plates. The substrate plates are then incubated at 70 °C for 20 minutes.

#### Example 4

##### Screening of Clones for Mannanase Activity

A solid phase screening assay was utilized as a primary screening method to test clones for  $\beta$ -mannanase activity.

5 A culture solution of the Y1090-*E. coli* host strain (Stratagene Cloning Systems, La Jolla, CA) was diluted to O.D.<sub>600</sub>=1.0 with NZY media. The amplified library from *Thermotoga maritima* lambda gtl1 library was diluted in SM (phage dilution buffer):  $5 \times 10^7$  pfu/ $\mu$ l diluted 1:1000 then 1:100 to  $5 \times 10^2$  pfu/ $\mu$ l. Then 8  $\mu$ l of phage dilution ( $5 \times 10^2$  pfu/ $\mu$ l) was plated in 200  $\mu$ l host cells. They were then incubated in 15 ml  
10 tubes at 37 °C for 15 minutes.

Approximately 4 ml of molten, LB top agarose (0.7%) at approximately 52 °C was added to each tube and the mixture was poured onto the surface of LB agar plates. The agar plates were then incubated at 37 °C for five hours. The plates were replicated and induced with 10 mM IPTG-soaked Duralon-UV™ nylon membranes (Stratagene  
15 Cloning Systems, La Jolla, CA) overnight. The nylon membranes and plates were marked with a needle to keep their orientation and the nylon membranes were then removed and stored at 4 °C.

An Azo-galactomannan overlay was applied to the LB plates containing the lambda plaques. The overlay contains 1% agarose, 50 mM potassium-phosphate buffer  
20 pH 7, 0.4% Azocarob-galactomannan. (Megazyme, Australia). The plates were incubated at 72 °C. The Azocarob-galactomannan treated plates were observed after 4 hours then returned to incubation overnight. Putative positives were identified by clearing zones on the Azocarob-galactomannan plates. Two positive clones were observed.

25 The nylon membranes referred to above, which correspond to the positive clones were retrieved, oriented over the plate and the portions matching the locations of the clearing zones for positive clones were cut out. Phage was eluted from the membrane cut-out portions by soaking the individual portions in 500  $\mu$ l SM (phage dilution buffer) and 25  $\mu$ l CHCl<sub>3</sub>.

### Example 5

#### Screening of Clones for Mannosidase Activity

A solid phase screening assay was utilized as a primary screening method to test clones for  $\beta$ -mannosidase activity.

5 A culture solution of the Y1090-*E. coli* host strain (Stratagene Cloning Systems, La Jolla, CA) was diluted to  $O.D_{600}=1.0$  with NZY media. The amplified library from AEPII 1a lambda gtl1 library was diluted in SM (phage dilution buffer):  $5 \times 10^7$  pfu/ $\mu$ l diluted 1:1000 then 1:100 to  $5 \times 10^2$  pfu/ $\mu$ l. Then 8  $\mu$ l of phage dilution ( $5 \times 10^2$  pfu/ $\mu$ l) was plated in 200  $\mu$ l host cells. They were then incubated in 15 ml  
10 tubes at 37 °C for 15 minutes.

Approximately 4 ml of molten, LB top agarose (0.7%) at approximately 52 °C was added to each tube and the mixture was poured onto the surface of LB agar plates. The agar plates were then incubated at 37 °C for five hours. The plates were replicated and induced with 10 mM IPTG-soaked Duralon-UV<sup>TM</sup> nylon membranes (Stratagene  
15 Cloning Systems, La Jolla, CA) overnight. The nylon membranes and plates were marked with a needle to keep their orientation and the nylon membranes were then removed and stored at 4 °C.

A p-nitrophenyl- $\beta$ -D-manno-pyranoside overlay was applied to the LB plates containing the lambda plaques. The overlay contains 1% agarose, 50 mM potassium-phosphate buffer pH 7, 0.4% p-nitrophenyl- $\beta$ -D-manno-pyranoside. (Megazyme,  
20 Australia). The plates were incubated at 72 °C. The p-nitrophenyl- $\beta$ -D-manno-pyranoside treated plates were observed after 4 hours then returned to incubation overnight. Putative positives were identified by clearing zones on the p-nitrophenyl- $\beta$ -D-manno-pyranoside plates. Two positive clones were observed.

25 The nylon membranes referred to above, which correspond to the positive clones were retrieved, oriented over the plate and the portions matching the locations of the clearing zones for positive clones were cut out. Phage was eluted from the membrane cut-out portions by soaking the individual portions in 500  $\mu$ l SM (phage dilution buffer) and 25  $\mu$ l  $CHCl_3$ .

### Example 6

#### Screening for Pullulanase Activity

Screening procedures for pullulanase protein activity may be assayed for as follows:

5 Substrate plates were provided by a standard plating procedure. Host cells are diluted to  $O.D._{600} = 1.0$  with NZY or appropriate media. In 15 ml tubes, inoculate 200  $\mu$ l diluted host cells with phage. Mix gently and incubate tubes at 37 °C for 15 min. Add approximately 3.5 ml LB top agarose (0.7%) is added to each tube and the mixture is plated, allowed to cool, and incubated at 37°C for about 28 hours. Overlays of 4.5  
10 mls of the following substrate are poured:

#### 100 ml total volume

0.5g	Red Pullulan Red (Megazyme, Australia)
1.0g	Agarose
5ml	Buffer (Tris-HCL pH 7.2 @ 75 °C)
15 2ml	5M NaCl
5ml	CaCl <sub>2</sub> (100mM)
85ml	dH <sub>2</sub> O

Plates are cooled at room temperature, and thenm incubated at 75°C for 2 hours.

Positives are observed as showing substrate degradation.

### Example 7

#### Screening for Endoglucanase Activity

Screening procedures for endoglucanase protein activity may be assayed for as follows:

25 1. The gene library is plated onto 6 LB/GelRite/0.1% CMC/NZY agar plates (~4,800 plaque forming units/plate) in E.coli host with LB agarose as top agarose. The plates are incubated at 37°C overnight.



2. Plates are chilled at 4°C for one hour.
3. The plates are overlaid with Duralon membranes (Stratagene) at room temperature for one hour and the membranes are oriented and lifted off the plates and stored at 4°C.
- 5 4. The top agarose layer is removed and plates are incubated at 37°C for ~3 hours.
5. The plate surface is rinsed with NaCl.
6. The plate is stained with 0.1% Congo Red for 15 minutes.
7. The plate is destained with 1M NaCl.
- 10 8. The putative positives identified on plate are isolated from the Duralon membrane (positives are identified by clearing zones around clones). The phage is eluted from the membrane by incubating in 500µl SM + 25µl CHCl<sub>3</sub> to elute.
9. Insert DNA is subcloned into any appropriate cloning vector and subclones are reassayed for CMCase activity using the following protocol:
  - 15 i) Spin 1ml overnight miniprep of clone at maximum speed for 3 minutes.
  - ii) Decant the supernatant and use it to fill "wells" that have been made in an LB/GelRite/0.1% CMC plate.
  - iii) Incubate at 37°C for 2 hours.
  - 20 iv) Stain with 0.1% Congo Red for 15 minutes.
  - v) Destain with 1M NaCl for 15 minutes.
  - vi) Identify positives by clearing zone around clone.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

25

**WHAT IS CLAIMED IS:**

1. An isolated polynucleotide selected from the group consisting of:
  - (a) SEQ ID NOS: 1-14 and 57-60;
  - (b) SEQ ID NOS: 1-14 and 57-60, wherein T can also be U;
  - (c) polynucleotide sequences complementary to SEQ ID NOS: 1-14 and 57-60;
  - (d) polynucleotide sequences which encode an amino acid sequence as set forth in SEQ ID NOS:15-28, and 61-64; and
  - (e) fragments of (a), (b), (c) or (d) that are at least 15 consecutive bases in length and that will selectively hybridize to DNA which encodes a polypeptide of SEQ ID NOS:15-28, and 61-64.
2. A vector comprising a polynucleotide of claim 1.
3. A host cell containing the vector of claim 2.
4. The method of claim 3, wherein the host cell is a eukaryotic cell.
5. The method of claim 3, wherein the host cell is a prokaryotic cell.
6. A method for producing a polypeptide comprising:
  - (a) culturing the host cells of claim 3;
  - (b) expressing from the host cell of claim 3 a polypeptide encoded by said polynucleotide; and
  - (c) isolating the polypeptide.

7. An enzyme selected from the group consisting of:
  - (a) an enzyme comprising an amino acid sequence set forth in SEQ ID NOS: 15-28 or 61-64; and
  - (b) an enzyme which comprises at least 30 consecutive amino acid residue as an enzyme of (a).
8. An enzyme of which at least a portion is coded for by a polynucleotide of claim 1, and which is selected from the group consisting of:
  - (a) an enzyme comprising an amino acid sequence which is at least 70% identical to an amino acid sequence selected from the group of amino acid sequences set forth in SEQ ID NOS: 15-28 or 61-64; and
  - (b) an enzyme which comprises at least 30 amino acid residues to the enzyme of (a).
9. A method for generating glucose from soluble cell oligosaccharides comprising contacting a sample containing oligosaccharides with an effective amount of an enzyme selected from the group consisting of an enzyme having the amino acid sequence set forth in SEQ ID NOS: 15-28, 61-63 and 64 such that glucose is produced.
10. The method of claim 9, wherein the sample is selected from the group consisting of dairy products, fruit juices, detergents, textiles, guar gum, animal feed, plant biomass and waste products.
11. The method of claim 9, wherein the oligosaccharide is selected from the group consisting of maltose, cellobiose, lactose, sucrose, raffinose, stachyose, verbascose, cellulose, starch, amylose, glycogen, disaccharides, polysaccharides and pullulan.



M11TL GLYCOSIDASE - 29C  
COMPLETE GENE SEQUENCE - 9/95

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1  TTT AAA GTT GTT AAA GAT TTT AGT ATA (22) TAT TTA TTT TTA TTT TTT GAA TTT GAA GAT 60
1 Met Lys Phe Pro Lys Asp Phe Met Ile Gly Tyr Ser Ser Ser Pro Phe Gln Phe Glu Ala 20
61  GTT ATT GTT (22) TTT GAG GAT GTT AAT AAT GAT TTT TTT GTA TTT GTT GAT GAT GTT GAT 120
21 Gly Ile Pro Gly Ser Glu Asp Pro Asn Ser Asp Trp Trp Val Trp Val His Asp Pro Glu 40
121 AAC ACA GCA GCT GGA CTA GTT AGT (22) GAT TTT GTT GAG AAC GTC CCA GAT TAT TTT AAT 180
41 Asn Thr Ala Ala Gly Leu Val Ser Gly Asp Phe Pro Glu Asn Gly Pro Gly Tyr Trp Asn 60
181 TTA AAC CAA AAT GAC CAC GAC GTT GTT GAG AAG CTG GCG GTT AAC ACT ATT AGA GTA (22) 240
61 Leu Asn Gln Asn Asp His Asp Leu Ala Glu Lys Leu Gly Val Asn Thr Ile Arg Val Gly 80
241 GTT GAG TGG AGT AGG ATT TTT CCA AAG CCA ACT TTC AAT GTT AAA GTC CCT GTA GAG AGA 300
81 Val Glu Trp Ser Arg Ile Phe Pro Lys Pro Thr Phe Asn Val Lys Val Pro Val Glu Arg 100
301 GAT GAG AAC GCG AGC ATT GTT CAC GTA GAT GTC GAT GAT AAA GCG GTT GAA AGA CTT GAT 360
101 Asp Glu Asn Gly Ser Ile Val His Val Asp Val Asp Asp Lys Ala Val Glu Arg Leu Asp 120
361 GAA TTA GCC AAC AAG CAG GCC GTA AAC CAT TAC GTA GAA ATG TAT AAA GAC TGG GTT GAA 420
121 Glu Leu Ala Asn Lys Glu Ala Val Asn His Tyr Val Glu Met Tyr Lys Asp Trp Val Glu 140
421 AGA GGT AGA AAA CTT ATA CTC AAT TTA TAC CAT TGG CCC CTG CCT CTC TGG CTT CAC AAC 480
141 Arg Gly Arg Lys Leu Ile Leu Asn Leu Tyr His Trp Pro Leu Pro Leu Trp Leu His Asn 160
481 CCA ATC ATG GTG AGA AGA ATG GCG CCG GAC AGA GCG CCC TCA GCG TGG CTT AAC GAG GAG 540
161 Pro Ile Met Val Arg Arg Met Gly Pro Asp Arg Ala Pro Ser Gly Trp Leu Asn Glu Glu 180
541 TCC GTG GTG GAG TTT GCC AAA TAC GCC GCA TAC ATT GCT TGG AAA ATG GCG GAG CTA CCT 600
181 Ser Val Val Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala Trp Lys Met Gly Glu Leu Pro 200
601 GTT ATG TGG AGC ACC ATG AAC GAA CCC AAC GTC GTT TAT GAG CAA GGA TAC ATG TTC GTT 660
201 Val Met Trp Ser Thr Met Asn Glu Pro Asn Val Val Tyr Glu Gln Gly Tyr Met Phe Val 220
661 AAA GGG GGT TTC CCA CCC GCG TAC TTG AGT TTG GAA GCT GCT GAT AAG GCC AGG AGA AAT 720
221 Lys Gly Gly Phe Pro Pro Gly Tyr Leu Ser Leu Glu Ala Ala Asp Lys Ala Arg Arg Asn 240
721 ATG ATC CAG GCT CAT GCA CCG GCC TAT GAC AAT ATT AAA CCG TTC AGT AAG AAA CCT GTT 780
241 Met Ile Gln Ala His Ala Arg Ala Tyr Asp Asn Ile Lys Arg Phe Ser Lys Lys Pro Val 260
781 GGA CTA ATA TAC GCT TTC CAA TGG TTC GAA CTA TTA GAG GGT CCA GCA GAA GTA TTT GAT 840
261 Gly Leu Ile Tyr Ala Phe Gln Trp Phe Glu Leu Leu Glu Gly Pro Ala Glu Val Phe Asp 280
841 AAG TTT AAG AGC TCT AAG TTA TAC TAT TTC ACA GAC ATA GTA TCG AAG GGT AGT TCA ATC 900
281 Lys Phe Lys Ser Ser Lys Leu Tyr Tyr Phe Thr Asp Ile Val Ser Lys Gly Ser Ser Ile 300
901 ATC AAT GTT GAA TAC AGG AGA GAT CTT GCC AAT AGG CTA GAC TGG TTG GCG GTT AAC TAC 960
301 Ile Asn Val Glu Tyr Arg Arg Asp Leu Ala Asn Arg Leu Asp Trp Leu Gly Val Asn Tyr 320
961 TAT AGC CGT TTA GTC TAC AAA ATC GTC GAT GAC AAA CCT ATA ATC CTG CAC GCG TAT GGA 1020
321 Tyr Ser Arg Leu Val Tyr Lys Ile Val Asp Asp Lys Pro Ile Ile Leu His Gly Tyr Gly 340
1021 TTC CTT TGT ACA CCT GCG GCG ATC AGC CCG GCT GAA AAT CCT TGT AGC GAT TTT GCG TGG 1080
341 Phe Leu Cys Thr Pro Gly Gly Ile Ser Pro Ala Glu Asn Pro Cys Ser Asp Phe Gly Trp 360
1081 GAG GTG TAT CCT GAA GGA CTC TAC CTA CTT CTA AAA GAA CTT TAC AAC CGA TAC GCG GTA 1140
361 Glu Val Tyr Pro Glu Gly Leu Tyr Leu Leu Lys Glu Leu Tyr Asn Arg Tyr Gly Val 380
1141 GAC TTG ATC GTG ACC GAG AAC GGT GTT TCA GAC AGC AGG GAT GCG TTG AGA CCG GCA TAC 1200
381 Asp Leu Ile Val Thr Glu Asn Gly Val Ser Asp Ser Arg Asp Ala Leu Arg Pro Ala Tyr 400
1201 CTG GTC TCG CAT GTT TAC AGC GTA TTT AAA GCG GGT AAC GAC GCG ATT CCC GTC AAA GCG 1260
401 Leu Val Ser His Val Tyr Ser Val Trp Lys Ala Ala Asn Glu Gly Ile Pro Val Lys Gly 420
1261 TAC CTT GAT TTT AGT TTG ACA GAT AAT TAT GAT TTT GTT GAT GCG TTT AGC GAT AAA TTT 1320
421 Tyr Leu His Trp Ser Leu Thr Asp Asn Tyr Glu Trp Ala Gln Gly Phe Arg Gln Lys Phe 440

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Figure 1a

1111 GGT TTA (TTT ATT GTT GAT TTT AAA ACT AAT AAA AAT TAT TTT TTT TTA AAT GAT TTA TTT 1100  
441 GGT TAA Val Met Val Asp Phe Lys Thr Lys Lys Arg Tyr Lys Arg Phe Ser Ala Lys Val 400  
1101 TTT TTT GAG ATT GGA AAT GAT AAT GGA ATA GTC GAT GAG GTA GAG GAT GGT AAT GTC ATT 1440  
461 Phe Arg Gln Ile Ala Thr His Asn Gly Ile Pro Asp Gln Lys Gln His Lys Thr Lys Ile 400  
1441 GAG TAA 1446  
481 Gln End 482

Figure 1b(Continued)

OC1/4 GLYCOSIDASE - 33G/B  
COMPLETE GENE SEQUENCE - 9/95

1 ATT ATA AGA AGG TCC GAT TTT TTA AAA GAT TTT ATC TTC GCA ACT GGT ATT GAA GCA TAC 60  
1 Met Ile Arg Arg Ser Asp Phe Pro Lys Asp Phe Ile Phe Gly Thr Ala Thr Ala Ala Tyr 20

61 TAG ATT GAA GGT CCA GCA AAC GAA GAT GCG ACA GGG CCA TCA ATT TAC GAT GTC TTT TCA 120  
21 Gln Ile Glu Gly Ala Ala Asn Glu Asp Gly Arg Gly Pro Ser Ile Trp Asp Val Phe Ser 40

121 CAC ACC CCT GGC AAA ACC CTC AAC GGT GAC ACA GCA GAC GTT GCG TTT GAC CAT TAT CAC 180  
41 His Thr Pro Gly Lys Thr Leu Asn Gly Asp Thr Gly Asp Val Ala Cys Asp His Tyr His 60

181 CGA TAC AAG GAA GAT ATC CAG CTG ATG AAA GAA ATA GGG TTA GAC CCT TAC AGG TTC TCT 240  
61 Arg Tyr Lys Glu Asp Ile Gln Leu Met Lys Glu Ile Gly Leu Asp Ala Tyr Arg Phe Ser 80

241 ATC TCC TGG CCC AGA ATT ATC CCA CAT GCG AAG AAC ATC AAC CAA AAG GGT GTG GAT TTC 300  
81 Ile Ser Trp Pro Arg Ile Met Pro Asp Gly Lys Asn Ile Asn Gln Lys Gly Val Asp Phe 100

301 TAC AAC AGA CTC GTT GAT GAG CTT TTG AAG AAT GAT ATC ATA CCA TTC GTA ACA CTC TAT 360  
101 Tyr Asn Arg Leu Val Asp Glu Leu Leu Lys Asn Asp Ile Ile Pro Phe Val Thr Leu Tyr 120

361 CAC TGG GAC TTA CCC TAC GCA CTT TAT GAA AAA GGT GGA TGG CTT AAC CCA GAT ATA GCG 420  
121 His Trp Asp Leu Pro Tyr Ala Leu Tyr Glu Lys Gly Gly Trp Leu Asn Pro Asp Ile Ala 140

421 CTC TAT TTC AGA GCA TAC GCA ACG TTT ATG TTC AAC GAA CTC GGT GAT CGT GTG AAA CAT 480  
141 Leu Tyr Phe Arg Ala Tyr Ala Thr Phe Met Phe Asn Glu Leu Gly Asp Arg Val Lys His 160

481 TGG ATT ACA CTG AAC GAA CCA TGG TGT TCT TCT TTC TCG GGT TAT TAC ACG GCA GAG CAT 540  
161 Trp Ile Thr Leu Asn Glu Pro Trp Cys Ser Ser Phe Ser Gly Tyr Tyr Thr Gly Glu His 180

541 GCC CCG GGT CAT CAA AAT TTA CAA GAA GCG ATA ATC GCG GCG CAC AAC CTC TTG AGG GAA 600  
181 Ala Pro Gly His Gln Asn Leu Gln Glu Ala Ile Ile Ala Ala His Asn Leu Leu Arg Glu 200

601 CAT GGA CAT GCG GTC CAG GCG TCC AGA GAA GAA GTA AAA GAT GCG GAA GTT GCG TTA ACC 660  
201 His Gly His Ala Val Gln Ala Ser Arg Glu Glu Val Lys Asp Gly Glu Val Gly Leu Thr 220

661 AAC GTT GTG ATG AAA ATA GAA CCG GCG GAT GCA AAA CCC GAA AGT TTC TTG GTC GCA AGT 720  
221 Asn Val Val Met Lys Ile Glu Pro Gly Asp Ala Lys Pro Glu Ser Phe Leu Val Ala Ser 240

721 CTT GTT GAT AAG TTC GTT AAT GCA TGG TCC CAT GAC CCT GTT GTT TTC GGA AAA TAT CCC 780  
241 Leu Val Asp Lys Phe Val Asn Ala Trp Ser His Asp Pro Val Val Phe Gly Lys Tyr Pro 260

781 GAA CAA GCA GTT GCA CTT TAT ACG GAA AAA GCG TTG CAA GTT CTC GAT AGC GAT ATG AAT 840  
261 Glu Glu Ala Val Ala Leu Tyr Thr Glu Lys Gly Leu Gln Val Leu Asp Ser Asp Met Asn 280

841 ATT ATT TCG ACT CCT ATA GAC TTC TTT GGT GTC AAT TAT TAC ACA AGA ACA CTT GTT GTT 900  
281 Ile Ile Ser Thr Pro Ile Asp Phe Phe Gly Val Asn Tyr Tyr Thr Arg Thr Leu Val Val 300

901 TTT GAT ATG AAC AAT CCT CTT GGA TTT TCG TAT CTT CAG GGA GAC CTT CCC AAA ACG GAG 960  
301 Phe Asp Met Asn Asn Pro Leu Gly Phe Ser Tyr Val Gln Gly Asp Leu Pro Lys Thr Glu 320

961 ATG GGA TGG GAA ATC TAC CCG CAG GGA TTA TTT GAT ATG CTG GTC TAT CTG AAG GAA AGA 1020  
321 Met Gly Trp Glu Ile Tyr Pro Gln Gly Leu Phe Asp Met Leu Val Tyr Leu Lys Glu Arg 340

1021 TAT AAA CTA CCA CTT TAT ATC ACA GAG AAC GCG ATG GCT GGA CCT GAT AAA TTG GAA AAC 1080  
341 Tyr Lys Leu Pro Leu Tyr Ile Thr Glu Asn Gly Met Ala Gly Pro Asp Lys Leu Glu Asn 360

1081 GGA AGA GTT CAT CAT AAT TAC CGA ATT GAA TAT TTG GAA AAG CAC TTT GAA AAA GCA CTT 1140  
361 Gly Arg Val His Asp Asn Tyr Arg Ile Glu Tyr Leu Glu Lys His Phe Glu Lys Ala Leu 380

1141 GAA CCA ATC AAT GCA GAT CTT GAT TTG AAA GGT TAC TTC ATT TGG TCT TTG ATG GAT AAC 1200  
381 Glu Ala Ile Asn Ala Asp Val Asp Leu Lys Gly Tyr Phe Ile Trp Ser Leu Met Asp Asn 400

1201 TTC GAA TGG GCG TCC GCA TAC TCC AAA CCG TTC GGT ATA ATC TAC GTA GAT TAC AAT ACC 1260  
401 Phe Glu Trp Ala Cys Gly Tyr Ser Lys Arg Phe Gly Ile Ile Tyr Val Asp Tyr Asn Thr 420

1261 CCA AAA AGG ATA TTG AAA GAT TCA GCG ATG TCG TTC AAG GAA TTT CTA AAA TCT TAA 1317  
421 Pro Lys Arg Ile Leu Lys Asp Ser Ala Met Trp Leu Lys Glu Phe Leu Lys Ser End 419

Figure 2

STAPHYLOTHERMUS MARINUS GLYCOSIDASE - 12G  
COMPLETE GENE SEQUENCE  
9/95

1 TTC ATA AGC TTT CCT GAT TAT TTC TTT TTT GGA AAT GGT ACA TCA TCG CAC CAG ATT GAG 60  
1 Met Ile Arg Phe Pro Asp Tyr Phe Leu Phe Gly Thr Ala Thr Ser Ser His Gln Ile Glu 20

61 CCT AAT AAC ATA TTT AAT GAT TCG TCG GAG TCG GAG ACT AAA GGC AGG ATT AAG GTG ACA 120  
21 Gly Asn Asn Ile Phe Asn Asp Trp Trp Glu Trp Glu Thr Lys Gly Arg Ile Lys Val Arg 40

121 TCG GGT AAG GCA TGT AAT CAT TCG GAA CTC TAT AAA GAA GAC ATA GAG CTT ATC GCT GAG 180  
41 Ser Gly Lys Ala Cys Asn His Trp Glu Leu Tyr Lys Glu Asp Ile Glu Leu Met Ala Glu 60

181 CTG GCA TAT AAT GCT TAT AGC TTC TCC ATA GAG TCG AGT AGA ATA TTT CCC AGA AAA GAT 240  
61 Leu Gly Tyr Asn Ala Tyr Arg Phe Ser Ile Glu Trp Ser Arg Ile Phe Pro Arg Lys Asp 80

241 CAT ATA GAT TAT GAG TCG CTT AAT AAG TAT AAG GAA ATA GTT AAT CTA CTT AGA AAA TAC 300  
81 His Ile Asp Tyr Glu Ser Leu Asn Lys Tyr Lys Glu Ile Val Asn Leu Leu Arg Lys Tyr 100

301 GGG ATA GAA CCT GTA ATC ACT CTT CAC CAC TTC ACA AAC CCG CAA TGG TTT ATG AAA ATT 360  
101 Gly Ile Glu Pro Val Ile Thr Leu His His Phe Thr Asn Pro Gln Trp Phe Met Lys Ile 120

361 GGT GGA TCG ACT AGC GAA GAG AAC ATA AAA TAT TTT ATA AAA TAT GTA GAA CTT ATA GCT 420  
121 Gly Gly Trp Thr Arg Glu Glu Asn Ile Lys Tyr Phe Ile Lys Tyr Val Glu Leu Ile Ala 140

421 TCC GAG ATA AAA GAC GTG AAA ATA TGG ATC ACT ATT AAT GAA CCA ATA ATA TAT GTT TTA 480  
141 Ser Glu Ile Lys Asp Val Lys Ile Trp Ile Thr Ile Asn Glu Pro Ile Ile Tyr Val Leu 160

481 CAA GCA TAT ATT TCC GGC GAA TGG CCA CCT GCA ATT AAA AAT TTA AAA ATA GCT GAT CAA 540  
161 Gln Gly Tyr Ile Ser Gly Glu Trp Pro Pro Gly Ile Lys Asn Leu Lys Ile Ala Asp Gln 180

541 GTA ACT AAG AAT CTT TTA AAA GCA CAT AAT GAA GCC TAT AAT ATA CTT CAT AAA CAC GGT 600  
181 Val Thr Lys Asn Leu Leu Lys Ala His Asn Glu Ala Tyr Asn Ile Leu His Lys His Gly 200

601 ATT GTA GGC ATA GCT AAA AAC ATG ATA GCA TTT AAA CCA GCA TCT AAT AGA GCA AAA GAC 660  
201 Ile Val Gly Ile Ala Lys Asn Met Ile Ala Phe Lys Pro Gly Ser Asn Arg Gly Lys Asp 220

661 ATT AAT ATT TAT CAT AAA GTC GAT AAA GCA TTC AAC TGG GCA TTT CTC AAC GGA ATA TTA 720  
221 Ile Asn Ile Tyr His Lys Val Asp Lys Ala Phe Asn Trp Gly Phe Leu Asn Gly Ile Leu 240

721 AGC GGA GAA CTA GAA ACT CTC CGT GGA AAA TAC CGA GTT GAG CCC GGA AAT ATT GAT TTC 780  
241 Arg Gly Glu Leu Glu Thr Leu Arg Gly Lys Tyr Arg Val Glu Pro Gly Asn Ile Asp Phe 260

781 ATA GGC ATA AAC TAT TAT TCA TCA TAT ATT GTA AAA TAT ACT TGG AAT CCT TTT AAA CTA 840  
261 Ile Gly Ile Asn Tyr Tyr Ser Ser Tyr Ile Val Lys Tyr Thr Trp Asn Pro Phe Lys Leu 280

841 CAT ATT AAA GTC GAA CCA TTA GAT ACA GGT CTA TGG ACA ACT ATG GGT TAC TGC ATA TAT 900  
281 His Ile Lys Val Glu Pro Leu Asp Thr Gly Leu Trp Thr Thr Met Gly Tyr Cys Ile Tyr 300

901 CCT AGA GGA ATA TAT GAA GTT GTA ATG AAA ACT CAT CAG AAA TAC GGC AAA GAA ATA ATC 960  
301 Pro Arg Gly Ile Tyr Glu Val Val Met Lys Thr His Glu Lys Tyr Gly Lys Glu Ile Ile 320

961 ATT ACA GAG AAC GGT GTT GCA GTA GAA AAT GAT GAA TTA AGC ATT TTA TCC ATT ATC AGC 1020  
321 Ile Thr Glu Asn Gly Val Ala Val Glu Asn Asp Glu Leu Arg Ile Leu Ser Ile Ile Arg 340

1021 CAC TTA CAA TAC TTA TAT AAA CCC ATG AAT GAA GGA GCA AAG GTC AAA GGA TAT TTC TAC 1080  
341 His Leu Gln Tyr Leu Tyr Lys Ala Met Asn Glu Gly Ala Lys Val Lys Gly Tyr Phe Tyr 360

1081 TCG ACC TTC ATG GAT AAT TTT GAG TCG GAT AAA GCA TTT AAC CAA AGC TTC GCA CTA GTA 1140  
361 Trp Ser Phe Met Asp Asn Phe Glu Trp Asp Lys Gly Phe Asn Gln Arg Phe Gly Leu Val 380

1141 GAA GTT GAT TAT AAG ACT TTT GAG AGA AAA CCT AGA AAA AGC GCA TAT GTA TAT ACT CAA 1200  
381 Glu Val Asp Tyr Lys Thr Phe Glu Arg Lys Pro Arg Lys Ser Ala Tyr Val Tyr Ser Gln 400

1201 ATA GCA CGT ACC AAG ACT ATA ACT GAT GAA TAC CTA GAA AAA TAT GGA TTA AAC AAC CTC 1260  
401 Ile Ala Arg Thr Lys Thr Ile Ser Asp Glu Tyr Leu Glu Lys Tyr Gly Leu Lys Asn Leu 420

1261 GAA TAA 1266  
421 Glu End 422

Figure 3



Thermococcus 9N1 Glycosidase -J18/0  
Complete gene sequence 9/95

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1 ATG CTA CCA GAA GGC TTT CTC TGG GGC GTG TCC CAG TCC GGC TTT CAG TTC GAG ATC GGC 60
1 Met Leu Pro Glu Gly Phe Leu Trp Gly Val Ser Gln Ser Gly Phe Gln Phe Glu Met Gly 20
61 GAC AAG CTC AGG AGG AAC ATT GAT CCG AAC ACA GAC TGG TGG AAG TGG GTC AGG GAT CCC 120
21 Asp Lys Leu Arg Arg Asn Ile Asp Pro Asn Thr Asp Trp Trp Lys Trp Val Arg Asp Pro 40
121 TTC AAC ATA AAG AGG GAA CTC CTC ACC GAC CTA CCC CAG CAG GCG ATA AAC AAC TAC 180
41 Phe Asn Ile Lys Arg Glu Leu Val Ser Gly Asp Leu Pro Glu Glu Gly Ile Asn Asn Tyr 60
181 GAA CTT TAC CAG AAG GAT CAC CGC CTC GCC AAG CAC CTC GGT CTG AAC GTT TAC AGG ATT 240
61 Glu Leu Tyr Glu Lys Asp His Arg Leu Ala Arg Asp Leu Gly Leu Asn Val Tyr Arg Ile 80
241 CGA ATA GAG TGG AGC AGG ATC TTT CCC TGG CCA ACC TGG TTT GTG GAG GTT GAC GTT GAG 300
81 Gly Ile Glu Trp Ser Arg Ile Phe Pro Trp Pro Thr Trp Phe Val Glu Val Asp Val Glu 100
301 CGG CAC AGC TAC GGA CTC GTG AAG GAC GTC AAA ATC GAT AAA GAC AGC CTC GAA GAG CTC 360
101 Arg Asp Ser Tyr Gly Leu Val Lys Asp Val Lys Ile Asp Lys Asp Thr Leu Glu Glu Leu 120
361 CAC CAG ATA GCG AAT CAT CAG CAC ATA GCG TAC TAC CGC CGC GTT ATA GAG CAC CTC AGG 420
121 Asp Glu Ile Ala Asn His Gln Glu Ile Ala Tyr Tyr Arg Arg Val Ile Glu His Leu Arg 140
421 CAG CTC GGC TTC AAG CTC ATC CTC AAC CTC AAC CAC TTC AGC CTC GCG CTC TGC CTT CAC 480
141 Glu Leu Gly Phe Lys Val Ile Val Asn Leu Asn His Phe Thr Leu Pro Leu Trp Leu His 160
481 GAT CGC ATA ATC GCG AGC CAG AAG GGT CTC ACC AAC GGT AGC ATT GCG TGG CTC GCG CAG 540
161 Asp Pro Ile Ile Ala Arg Glu Lys Ala Leu Thr Asn Gly Arg Ile Gly Trp Val Gly Gln 180
541 GAG AGC CTC GTG CAC TTC GCG AAG TAC GCG GGT TAC ATC GCG AAC GCA CTC GCG GAC CTC 600
181 Glu Ser Val Val Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala Asn Ala Leu Gly Asp Leu 200
601 GTT CAT ATG TGG AGC ACC TTC AAC GAG CGC ATG GTC GTT GTG CAG CTC GGT TAC CTC GCG 660
201 Val Asp Met Trp Ser Thr Phe Asn Glu Pro Met Val Val Val Glu Leu Gly Tyr Leu Ala 220
661 CCC TAC TCC GGC TTT CCG CGC GCG GTT ATG AAC CCC GAG GCG GCA AAG CTG GCA ATC CTC 720
221 Pro Tyr Ser Gly Phe Pro Pro Gly Val Met Asn Pro Glu Ala Ala Lys Leu Ala Ile Leu 240
721 AAC ATG ATA AAC GGC CAC GCA CTC GCG TAC AAG ATG ATA AAG AAG TTC GAC AGG GTA AAG 780
241 Asn Met Ile Asn Ala His Ala Leu Ala Tyr Lys Met Ile Lys Lys Phe Asp Arg Val Lys 260
781 GGC GAT AAG GAT TCC GCG TCC GAG GCG GAG GTC GCG ATA ATT TAC AAC AAC ATA GCG GTT 840
261 Ala Asp Lys Asp Ser Arg Ser Glu Ala Glu Val Gly Ile Ile Tyr Asn Asn Ile Gly Val 280
841 GGC TAT CCA TAC CAC TCC AAC GAC CCA AAG CAC GTG AAA GGT GCA GAA AAC CAC AAC TAC 900
281 Ala Tyr Pro Tyr Asp Ser Asn Asp Pro Lys Asp Val Lys Ala Ala Glu Asn Asp Asn Tyr 300
901 TTC CAC AGC GCG CTC TTC TTC GAC GCA ATC CAC AAG GCG AAG CTC AAC ATC CAG TTC CAC 960
301 Phe His Ser Gly Leu Phe Phe Asp Ala Ile His Lys Gly Lys Leu Asn Ile Glu Phe Asp 320
961 GGT CAC ACC TTC GTC AAA GTT CCG CAT CTC AGC GCG AAC CAC TCG ATA GCG GTT AAC TAC 1020
321 Gly Glu Thr Phe Val Lys Val Arg His Leu Arg Gly Asn Asp Trp Ile Gly Val Asn Tyr 340
1021 TAC ACC AGA GAA GTC GTC AGG TAT TCG GAG CCG AAC TTC CCG AGC ATA CCC CTG ATA TCC 1080
341 Tyr Thr Arg Glu Val Val Arg Tyr Ser Glu Pro Lys Phe Pro Ser Ile Pro Leu Ile Ser 360
1081 TTC CCG GCA GTT CAC AAC TAC GCG TAC GCG TCG AGG CCG GCG ACT TCT TCC GCG CAC CGA 1140
361 Phe Arg Gly Val His Asn Tyr Gly Tyr Ala Cys Arg Pro Gly Ser Ser Ser Ala Asp Gly 380
1141 AGC CCC GTA AGC GAC ATC GCG TGG GAG ATC TAT CCG GAG GCG ATC TAC GAC TCG ATA AGA 1200
381 Arg Pro Val Ser Asp Ile Gly Trp Glu Ile Tyr Pro Glu Gly Ile Tyr Asp Ser Ile Arg 400
1201 GAG GCG AAC AAA TAC GCG CTC CCG GTT TAC GTC ACC GAA AAC GCA ATA CCC CAT TCA ACT 1260
401 Glu Ala Asn Lys Tyr Gly Val Pro Val Tyr Val Thr Glu Asn Gly Ile Ala Asp Ser Thr 420
1261 CAC ACC CTG CCG CCG TAC TAC CTC GCG ACC CAT CTA CCG AAG ATT CAG GAG CCG TAC CAG 1320
421 Asp Thr Leu Arg Pro Tyr Tyr Leu Ala Ser His Val Ala Lys Ile Glu Glu Ala Tyr Glu 440

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Figure 4a

1321	CCG GCT TAC GAC GTC ACC GGC TAC CTC TAC TGG GCG CTG ACC GAC AAC TAC GAG TGG GCC	1380
441	Ala Gly Tyr Asp Val Arg Gly Tyr Leu Tyr Trp Ala Leu Thr Asp Asn Tyr Glu Trp Ala	460
1381	CTC GCT TTC AGG ATC AGC TTC GGC CTC TAT AAA CTG GAT CTC ATA ACC AAG CAC ACA ACA	1440
461	Leu Gly Phe Arg Met Arg Phe Gly Leu Tyr Lys Val Asp Leu Ile Thr Lys Glu Arg Thr	480
1441	CCG CCG CAG GAA ACC GTA AAG GTT TAT ACC CCG ATC CTC CAG AAC AAC CCA GTC AOC AAC	1500
481	Pro Arg Glu Glu Ser Val Lys Val Tyr Arg Gly Ile Val Glu Asn Asn Gly Val Ser Lys	500
1501	GAA ATC CCG CAG AAG TTC CCA CTT GCG TGA	1530
501	Glu Ile Arg Glu Lys Phe Gly Leu Gly End	510

Figure 4b(Continued)

1	ATG	GAA	ACG	ATC	GAT	GAA	ATT	CTC	TCT	CAG	TTA	ACT	ACA	GAG	GAA	AAC	CTG	AAC	CTC	GTT	81
1	Met	Glu	Arg	Ile	Asp	Glu	Ile	Leu	Ser	Gln	Leu	Thr	Thr	Glu	Glu	Lys	Val	Lys	Leu	Val	21
61	GTG	GGG	GTT	GGT	CTT	CCA	GGA	CTT	TTT	GGG	AAC	CCA	CAT	TCC	AGA	CTG	GGG	GCT	GCG	GCT	120
21	Val	Gly	Val	Gly	Ile	Pro	Gly	Leu	Phe	Gly	Asn	Pro	His	Ser	Arg	Val	Ala	Gly	Ala	Ala	40
121	GGA	GAA	ACA	CAT	CCC	GTT	CCA	AGA	CTT	GGA	ATT	CCT	GCG	TTT	GTC	CTG	GCA	GAT	GGT	CCC	180
41	Gly	Glu	Thr	His	Pro	Val	Pro	Arg	Leu	Gly	Ile	Pro	Ala	Phe	Val	Leu	Ala	Asp	Gly	Pro	60
181	GCA	GGA	CTC	AGA	ATA	AAT	CCC	ACA	AGG	GAA	AAC	GAT	GAA	AAC	ACT	TAC	TAC	ACG	ACG	GCA	240
61	Ala	Gly	Leu	Arg	Ile	Asn	Pro	Thr	Arg	Glu	Asn	Asp	Glu	Asn	Thr	Tyr	Tyr	Thr	Thr	Ala	80
241	TTT	CCC	GTT	GAA	ATC	ATG	CTC	GCT	TCT	ACC	TGG	AAC	AGA	GAC	CTT	CTG	GAA	GAA	GTG	GGA	300
81	Phe	Pro	Val	Glu	Ile	Met	Leu	Ala	Ser	Thr	Trp	Asn	Arg	Asp	Leu	Leu	Glu	Glu	Val	Gly	100
301	AAA	GCC	ATG	GGA	GAA	GAA	GTT	AGG	GAA	TAC	GGT	GTC	GAT	GTG	CTT	CTT	GCA	CCT	GCG	ATG	360
101	Lys	Ala	Met	Gly	Glu	Glu	Val	Arg	Glu	Tyr	Gly	Val	Asp	Val	Leu	Leu	Ala	Pro	Ala	Met	120
361	AAC	ATT	CAC	AGA	AAC	CCT	CTT	TGT	GGA	AGG	AAT	TTC	GAG	TAC	TAC	TCA	GAA	GAT	CCT	GTC	420
121	Asn	Ile	His	Arg	Asn	Pro	Leu	Cys	Gly	Arg	Asn	Phe	Glu	Tyr	Tyr	Ser	Glu	Asp	Pro	Val	140
421	CTT	TCC	GGT	GAA	ATG	GCT	TCA	GCC	TTT	GTC	AAG	GGA	GTT	CAA	TCT	CAA	GGG	GTG	GGA	GCC	480
141	Leu	Ser	Gly	Glu	Met	Ala	Ser	Ala	Phe	Val	Lys	Gly	Val	Gln	Ser	Gln	Gly	Val	Gly	Ala	160
481	TGC	ATA	AAA	CAC	TTT	GTC	GCG	AAC	AAC	CAG	GAA	ACG	AAC	AGG	ATG	GTA	GTG	GAC	ACG	ATC	540
161	Cys	Ile	Lys	His	Phe	Val	Ala	Asn	Asn	Gln	Glu	Thr	Asn	Arg	Met	Val	Val	Asp	Thr	Ile	180
541	GTG	TCC	GAG	CGA	GCC	CTC	AGA	GAA	ATA	TAT	CTG	AAA	GGT	TTT	GAA	ATT	GCT	GTC	AAG	AAA	600
181	Val	Ser	Glu	Arg	Ala	Leu	Arg	Glu	Ile	Tyr	Leu	Lys	Gly	Phe	Glu	Ile	Ala	Val	Lys	Lys	200
601	GCA	AGA	CCC	TGG	ACC	GTG	ATG	AGC	GCT	TAC	AAC	AAA	CTG	AAT	GGA	AAA	TAC	TGT	TCA	CAG	660
201	Ala	Arg	Pro	Trp	Thr	Val	Met	Scr	Ala	Tyr	Asn	Lys	Leu	Asn	Gly	Lys	Tyr	Cys	Ser	Gln	220
661	AAC	GAA	TGG	CTT	TTG	AAG	AAG	GTT	CTC	AGG	GAA	GAA	TGG	GGA	TTT	GGC	GGT	TTC	GTG	ATG	720
221	Asn	Glu	Trp	Leu	Leu	Lys	Lys	Val	Leu	Arg	Glu	Glu	Trp	Gly	Phe	Gly	Gly	Phe	Val	Met	240
721	AGC	GAC	TGG	TAC	GCG	GGA	GAC	AAC	CCT	GTA	GAA	CAG	CTC	AAG	GCC	GGA	AAC	GAT	ATG	ATC	780
241	Ser	Asp	Trp	Tyr	Ala	Gly	Asp	Asn	Pro	Val	Glu	Gln	Leu	Lys	Ala	Gly	Asn	Asp	Met	Ile	260
781	ATG	CCT	GGG	AAA	GCG	TAT	CAG	GTG	AAC	ACA	GAA	AGA	AGA	GAT	GAA	ATA	GAA	GAA	ATC	ATG	840
261	Met	Pro	Gly	Lys	Ala	Tyr	Gln	Val	Asn	Thr	Glu	Arg	Arg	Asp	Glu	Ile	Glu	Glu	Ile	Met	280
841	GAG	GCG	TTG	AAG	GAG	GGA	AAA	TTG	AGT	GAG	GAG	GTT	CTC	GAT	GAG	TGT	GTG	AGA	AAC	ATT	900
281	Glu	Ala	Leu	Lys	Glu	Gly	Lys	Leu	Ser	Glu	Glu	Val	Leu	Asp	Glu	Cys	Val	Arg	Asn	Ile	300
901	CTC	AAA	GTT	CTT	GTG	AAC	GCG	CCT	TCC	TTC	AAA	GGG	TAC	AGG	TAC	TCA	AAC	AAG	CCG	GAT	960
301	Leu	Lys	Val	Leu	Val	Asn	Ala	Pro	Ser	Phe	Lys	Gly	Tyr	Arg	Tyr	Ser	Asn	Lys	Pro	Asp	320
961	CTC	GAA	TCT	CAC	GCG	GAA	GTC	GCC	TAC	GAA	GCA	GGT	GCG	GAG	GGT	GTT	GTC	CTT	CTT	GAG	1020
321	Leu	Glu	Ser	His	Ala	Glu	Val	Ala	Tyr	Glu	Ala	Gly	Ala	Glu	Gly	Val	Val	Leu	Leu	Glu	340
1021	AAC	AAC	GGT	GTT	CTT	CCG	TTC	GAT	GAA	AAT	ACC	CAT	GTC	GCC	GTC	TTT	GGC	ACC	GGT	CAA	1080
341	Asn	Asn	Gly	Val	Leu	Pro	Phe	Asp	Glu	Asn	Thr	His	Val	Ala	Val	Phe	Gly	Thr	Gly	Gln	360
1081	ATC	GAA	ACA	ATA	AAG	GGA	GGA	ACG	GGA	AGT	GGA	GAC	ACC	CAT	CCG	AGA	TAC	ACG	ATC	TCT	1140
361	Ile	Glu	Thr	Ile	Lys	Gly	Gly	Thr	Gly	Ser	Gly	Asp	Thr	His	Pro	Arg	Tyr	Thr	Ile	Ser	380
1141	ATC	CTT	GAA	GGT	ATA	AAA	GAA	AGA	AAC	ATG	AAG	TTT	GAC	GAA	GAA	CTC	GCT	TCT	ACT	TAT	1200
381	Ile	Leu	Glu	Gly	Ile	Lys	Glu	Arg	Asn	Met	Lys	Phe	Asp	Glu	Glu	Leu	Ala	Ser	Thr	Tyr	400

Figure:5a

1201	GAG	GAG	TAC	ATA	AAA	AAG	ATG	AGA	GAA	ACA	GAG	GAA	TAT	AAA	ATG	AGA	ATG	GAC	TCT	TGG	1260
401	Glu	Glu	Tyr	Ile	Lys	Lys	Met	Arg	Glu	Thr	Glu	Glu	Tyr	Lys	Pro	Arg	Thr	Asp	Ser	Trp	420
1261	GGA	ACG	GTC	ATA	AAA	CCG	AAA	CTC	CCA	GAG	AAT	TTC	CTC	TCA	GAA	AAA	GAG	ATA	AAG	AAA	1320
421	Gly	Thr	Val	Ile	Lys	Pro	Lys	Leu	Pro	Glu	Asn	Phe	Leu	Ser	Glu	Lys	Glu	Ile	Lys	Lys	440
1321	CCT	CCA	AAG	AAA	AAC	GAT	GTT	GCA	GTT	GTT	GTG	ATC	AGT	AGG	ATC	TCC	GCT	GAG	GGA	TAC	1380
441	Pro	Pro	Lys	Lys	Asn	Asp	Val	Ala	Val	Val	Val	Ile	Ser	Arg	Ile	Ser	Gly	Glu	Gly	Tyr	460
1381	GAC	AGA	AAG	CCG	GTG	AAA	GGT	GAC	TTC	TAC	CTC	TCC	GAT	GAC	GAG	CTG	GAA	CTC	ATA	AAA	1440
461	Asp	Arg	Lys	Pro	Val	Lys	Gly	Asp	Phe	Tyr	Leu	Ser	Asp	Asp	Glu	Leu	Glu	Leu	Ile	Lys	480
1441	ACC	GTC	TCG	AAA	GAA	TTC	CAC	GAT	CAG	GGT	AAG	AAA	GTT	GTG	GTT	CTT	CTG	AAC	ATC	GGA	1500
481	Thr	Val	Ser	Lys	Glu	Phe	His	Asp	Gln	Gly	Lys	Lys	Val	Val	Val	Leu	Leu	Asn	Ile	Gly	300
1501	AGT	CCC	ATC	GAA	GTC	GCA	AGC	TGG	AGA	GAC	CTT	GTG	GAT	GGA	ATT	CTT	CTC	GTC	TGG	CAG	1560
501	Ser	Pro	Ile	Glu	Val	Ala	Ser	Trp	Arg	Asp	Leu	Val	Asp	Gly	Ile	Leu	Leu	Val	Trp	Gln	320
1561	GCG	GGA	CAG	GAG	ATG	GGA	AGA	ATA	GTG	CCC	GAT	GTT	CTT	GTG	GGA	AAG	ATT	AAT	CCC	TCC	1620
521	Ala	Gly	Gln	Glu	Met	Gly	Arg	Ile	Val	Ala	Asp	Val	Leu	Val	Gly	Lys	Ile	Asn	Pro	Ser	340
1621	GGA	AAA	CTT	CCA	ACG	ACC	TTC	CCG	AAG	GAT	TAC	TCG	GAC	GTT	CCA	TCC	TGG	ACG	TTC	CCA	1680
541	Gly	Lys	Leu	Pro	Thr	Thr	Phe	Pro	Lys	Asp	Tyr	Ser	Asp	Val	Pro	Ser	Trp	Thr	Phe	Pro	360
1681	GGA	GAG	CCA	AAG	GAC	AAT	CCG	CAA	AGA	GTG	GTG	TAC	GAG	GAA	GAC	ATC	TAC	GTG	GGA	TAC	1740
561	Gly	Glu	Pro	Lys	Asp	Asn	Pro	Gln	Arg	Val	Val	Tyr	Glu	Glu	Asp	Ile	Tyr	Val	Gly	Tyr	380
1741	AGG	TAC	TAC	GAC	ACC	TTC	GGT	GTG	GAA	CCT	CCC	TAC	GAA	TTC	GGC	TAC	GGC	CTC	TCT	TAC	1800
581	Arg	Tyr	Tyr	Asp	Thr	Phe	Gly	Val	Glu	Pro	Ala	Tyr	Glu	Phe	Gly	Tyr	Gly	Leu	Ser	Tyr	600
1801	ACA	AAG	TTT	GAA	TAC	AAA	GAT	TTA	AAA	ATC	GCT	ATC	GAC	GGT	GAG	ACG	CTC	AGA	GTG	TCG	1860
601	Thr	Lys	Phe	Glu	Tyr	Lys	Asp	Leu	Lys	Ile	Ala	Ile	Asp	Gly	Glu	Thr	Leu	Arg	Val	Ser	620
1861	TAC	ACG	ATC	ACA	AAC	ACT	GGG	GAC	AGA	GCT	GGA	AAG	GAA	GTC	TCA	CAG	GTC	TAC	ATC	AAA	1920
621	Tyr	Thr	Ile	Thr	Asn	Thr	Gly	Asp	Arg	Ala	Gly	Lys	Glu	Val	Ser	Gln	Val	Tyr	Ile	Lys	640
1921	GCT	CCA	AAA	GGA	AAA	ATA	GAC	AAA	CCC	TTC	CAG	GAG	CTG	AAA	GCG	TTT	CAC	AAA	ACA	AAA	1980
641	Ala	Pro	Lys	Gly	Lys	Ile	Asp	Lys	Pro	Phe	Gln	Glu	Leu	Lys	Ala	Phe	His	Lys	Thr	Lys	660
1981	CTT	TTG	AAC	CCG	GGT	GAA	TCA	GAA	GAA	ATC	TCC	TTG	GAA	ATT	CCT	CTC	AGA	GAT	CTT	GCG	2040
661	Leu	Leu	Asn	Pro	Gly	Glu	Ser	Glu	Glu	Ile	Ser	Leu	Glu	Ile	Pro	Leu	Arg	Asp	Leu	Ala	680
2041	AGT	TTC	GAT	GGG	AAA	GAA	TGG	GTT	GTC	GAG	TCA	GGA	GAA	TAC	GAG	GTC	AGG	GTC	GGT	GCA	2100
681	Ser	Phe	Asp	Gly	Lys	Glu	Trp	Val	Val	Glu	Ser	Gly	Glu	Tyr	Glu	Val	Arg	Val	Gly	Ala	700
2101	TCT	TCG	AGG	GAT	ATA	AGG	TTG	AGA	GAT	ATT	TTT	CTG	GTT	GAG	GGA	GAG	AAG	AGA	TTC	AAA	2160
701	Ser	Ser	Arg	Asp	Ile	Arg	Leu	Arg	Asp	Ile	Phe	Leu	Val	Glu	Gly	Glu	Lys	Arg	Phe	Lys	2180
2161	CCA	TGA	2166																		
721	Pro	End	722																		

Figure 5b(Continued)

THERMOCOCCUS AEDII12RA GLYCOSIDASE (188/G)  
COMPLETE GENE SEQUENCE - 9/95

1	ATC ATC CAC TCC CCG GTT AAA CCG ATT ATA TCT CAG CCT CCC GGC ATA ALT ATC ACA ATA	60
1	Met Ile His Cys Pro Val Lys Gly Ile Ile Ser Glu Ala Arg Gly Ile Thr Ile Thr Ile	20
61	GAT TTA ACT TTT CAA GGC CAA ATA AAT AAT TTC CTC AAT GCT ATC ATT GTC TTT CCG CAG	120
21	Asp Leu Ser Phe Gln Gly Gln Ile Asn Asn Leu Val Asn Ala Met Ile Val Phe Pro Glu	40
121	TTC TTC CTC TTT GGA ACC GGC ACA TCT TCT CAT CAG ATC GAG GGA CAT AAT AAA TCG AAC	180
41	Phe Phe Leu Phe Gly Thr Ala Thr Ser Ser His Gln Ile Glu Gly Asp Asn Lys Trp Asn	60
181	CAC TGG TCG TAT TAT CAG GAG ATA GGT AAG CTC CCC TAC AAA TCC GGT AAA CCC TCG AAT	240
61	Asp Trp Trp Tyr Tyr Glu Glu Ile Gly Lys Leu Pro Tyr Lys Ser Gly Lys Ala Cys Asn	80
241	CAC TGG GAG CTT TAC ACC GAA GAT ATA GAG CTA ATC GCA CAG CTC GGC TAC AAT GCC TAC	300
81	His Trp Glu Leu Tyr Arg Glu Asp Ile Glu Leu Met Ala Gln Leu Gly Tyr Asn Ala Tyr	100
301	CGC TTT TCG ATA GAG TGG ACC CGT CTC TTC CCG GAA GAG GGC AAA TTC AAT GAA GAA GCC	360
101	Arg Phe Ser Ile Glu Trp Ser Arg Leu Phe Pro Glu Glu Gly Lys Phe Asn Glu Glu Ala	120
361	TTC AAC CCG TAC CGT GAA ATA ATT GAA ATC CTC CTT GAG AAG GCG ATT ACT CCA AAC GTT	420
121	Phe Asn Arg Tyr Arg Glu Ile Ile Glu Ile Leu Leu Glu Lys Gly Ile Thr Pro Asn Val	140
421	ACA CTG CAC CAC TTC ACA TCA CCG CTG TGG TTC ATG CCG AAG GGA GGC TTT TCG AAG GAA	480
141	Thr Leu His His Phe Thr Ser Pro Leu Trp Phe Met Arg Lys Gly Gly Phe Leu Lys Glu	160
481	GAA AAC CTC AAG TAC TGG GAG CAG TAC GTT GAT AAA GCC GCG CAG CTC CTC AAG GGA CTC	540
161	Glu Asn Leu Lys Tyr Trp Glu Gln Tyr Val Asp Lys Ala Ala Glu Leu Leu Lys Gly Val	180
541	AAG CTT GTA CCT ACA TTC AAC GAG CCG ATG GTC TAT GTT ATG ATG GGC TAC CTC ACA GCC	600
181	Lys Leu Val Ala Thr Phe Asn Glu Pro Met Val Tyr Val Met Met Gly Tyr Leu Thr Ala	200
601	TAC TGG CCG CCC TTC ATC AAG AGT CCC TTT AAA GCC TTT AAA GTT GCC GCA AAC CTC CTT	660
201	Tyr Trp Pro Pro Phe Ile Lys Ser Pro Phe Lys Ala Phe Lys Val Ala Ala Asn Leu Leu	220
661	AAG GCC CAT GCA ATG GCA TAT GAT ATC CTC CAT GGT AAC TTT GAT GTG GCG ATA GTT AAA	720
221	Lys Ala His Ala Met Ala Tyr Asp Ile Leu His Gly Asn Phe Asp Val Gly Ile Val Lys	240
721	AAC ATC CCC ATA ATG CTC CCT GCA ACC AAC AGA GAG AAA GAC GTA GAA GCT GCC CAA AAG	780
241	Asn Ile Pro Ile Met Leu Pro Ala Ser Asn Arg Glu Lys Asp Val Glu Ala Ala Gln Lys	260
781	CGG GAT AAC CTC TTT AAC TGG AAC TTC CTT GAT GCA ATA TGG ACC GGA AAA TAT AAA GGA	840
261	Ala Asp Asn Leu Phe Asn Trp Asn Phe Leu Asp Ala Ile Trp Ser Gly Lys Tyr Lys Gly	280
841	GCT TTT GGA ACT TAC AAA ACT CCA GAA ACC GAT GCA GAC TTC ATA GCG ATA AAC TAC TAC	900
281	Ala Phe Gly Thr Tyr Lys Thr Pro Glu Ser Asp Ala Asp Phe Ile Gly Ile Asn Tyr Tyr	300
901	ACA GCC AGC GAG GTA AGG CAT ACC TGG AAT CCG CTA AAG TTT TTC TTC GAT GCC AAG CTT	960
301	Thr Ala Ser Glu Val Arg His Ser Trp Asn Pro Leu Lys Phe Phe Phe Asp Ala Lys Leu	320
961	GCA GAC TTA ACC GAG AGA AAA ACA GAT ATG GGT TGG AGT GTC TAT CCA AAG GGC ATA TAC	1020
321	Ala Asp Leu Ser Glu Arg Lys Thr Asp Met Gly Trp Ser Val Tyr Pro Lys Gly Ile Tyr	340
1021	GAA GCT ATA GCA AAG GTT TCA CAC TAC GGA AAG CCA ATG TAC ATC ACG GAA AAC GGC ATA	1080
341	Glu Ala Ile Ala Lys Val Ser His Tyr Gly Lys Pro Met Tyr Ile Thr Glu Asn Gly Ile	360
1081	GCT ACC TTA CAC GAT GAG TGG AGC ATA GAG TTT ATC ATC CAG CAC CTC CAG TAC GTT CAC	1140
361	Ala Thr Leu Asp Asp Glu Trp Arg Ile Glu Phe Ile Ile Gln His Leu Gln Tyr Val His	380
1141	AAA GCC TTA AAC GAT GGC TTT GAC TTG AGA GGC TAC TTC TAT TGG TCT TTT ATC GAT AAC	1200
381	Lys Ala Leu Asn Asp Gly Phe Asp Leu Arg Gly Tyr Phe Tyr Trp Ser Phe Met Asp Asn	400
1201	TTC GAG TGG GCT GAG CCG TTT AGA CCA CCG TTT GCG CTG CTC GAG GTG GAC TAC ACG ACC	1260
401	Phe Glu Trp Ala Glu Gly Phe Arg Pro Arg Phe Gly Leu Val Glu Val Asp Tyr Thr Thr	420
1261	TTC AAG ACC ACA CCG AGA AAG AGT GCT TAC ATA TAT GGA GAA ATT GCA AGG GAA AAG AAA	1320
421	Phe Lys Arg Arg Pro Arg Lys Ser Ala Tyr Ile Tyr Gly Glu Ile Ala Arg Glu Lys Lys	440
1321	ATA AAA CAC GAA CTC CTC GCA AAG TAT GCG CTT CCC GAG CTA TGA	1365
441	Ile Lys Asp Glu Leu Leu Ala Lys Tyr Gly Leu Pro Glu Leu End	455

Figure 6

THERMOCOCCUS CHITONOPHAGUS GLYCOSIDASE - 22G  
COMPLETE SEQUENCE - 9/95

1	TTC CTT CCA GAG AAC TTT CTC TGG CGA GTT TCA CAG TCC CGA TTC CAG TTT GAA ATC GUC	60
1	Met Leu Pro Glu Asn Phe Leu Trp Gly Val Ser Gln Ser Gly Phe Gln Phe Glu Met Gly	20
61	GAC AGA CTC AGC AGC CAC ATT GAT CCA AAC ACA CAT TGG TGG TAC TGG CTA AGA GAT GAA	120
21	Asp Arg Leu Arg Arg His Ile Asp Pro Asn Thr Asp Trp Trp Tyr Trp Val Arg Asp Glu	40
121	TAT AAT ATC AAA AAA GCA CTA GTA AGT CGG GAT CTT CCC GAA GAC GGT ATA AAT TCA TAT	180
41	Tyr Asn Ile Lys Lys Gly Leu Val Ser Gly Asp Leu Pro Glu Asp Gly Ile Asn Ser Tyr	60
181	GAA TTA TAT GAG AGA GAC CAA GAA ATT GCA AAG GAT TTA GGC CTC AAC ACA TAT AGG ATC	240
61	Glu Leu Tyr Glu Arg Asp Gln Glu Ile Ala Lys Asp Leu Gly Leu Asn Thr Tyr Arg Ile	80
241	GGA ATT GAA TGG AGC AGA GTA TTT CCA TGG CCA ACG ACT TTT GTC GAC CTG GAG TAT GAA	300
81	Gly Ile Glu Trp Ser Arg Val Phe Pro Trp Pro Thr Thr Phe Val Asp Val Glu Tyr Glu	100
301	ATT GAT CAG TCT TAC GCG TTG GTA AAG GAT GTG AAG ATT TCT AAA GAC GCA TTA GAA AAA	360
101	Ile Asp Glu Ser Tyr Gly Leu Val Lys Asp Val Lys Ile Ser Lys Asp Ala Leu Glu Lys	120
361	CTT GAT GAA ATC GCT AAC CAA AGG GAA ATA ATA TAT TAT AGG AAC CTA ATA AAT TCC CTA	420
121	Leu Asp Glu Ile Ala Asn Gln Arg Glu Ile Ile Tyr Tyr Arg Asn Leu Ile Asn Ser Leu	140
421	AGA AAG AGG GGT TTT AAG GTA ATA CTA AAC CTA AAT CAT TTT ACC CTC CCA ATA TGG CTT	480
141	Arg Lys Arg Gly Phe Lys Val Ile Leu Asn Leu Asn His Phe Thr Leu Pro Ile Trp Leu	160
481	CAT GAT CCT ATC GAA TCT AGA GAA AAA CCC CTG ACC AAT AAG AGA AAC GGA TGG GTA AGC	540
161	His Asp Pro Ile Glu Ser Arg Glu Lys Ala Leu Thr Asn Lys Arg Asn Gly Trp Val Ser	180
541	GAA AGG AGT GTT ATA GAG TTT GCA AAA TTT GCG GCG TAT TTA GCA TAT AAA TTC GCA GAC	600
181	Glu Arg Ser Val Ile Glu Phe Ala Lys Phe Ala Ala Tyr Leu Ala Tyr Lys Phe Gly Asp	200
601	ATA GTA GAC ATG TGG AGC ACA TTT AAT GAA CCT ATG GTG GTC CCC GAG TTG GCG TAT TTA	660
201	Ile Val Asp Met Trp Ser Thr Phe Asn Glu Pro Met Val Val Ala Glu Leu Gly Tyr Leu	220
661	CCC CCA TAC TCA GCA TTC CCC CCG GCA GTC ATG AAT CCA GAA GCA GCA AAG TTA GTT ATG	720
221	Ala Pro Tyr Ser Gly Phe Pro Pro Gly Val Met Asn Pro Glu Ala Ala Lys Leu Val Met	240
721	CTA CAT ATG ATA AAC GCC CAT GCT TTA GCA TAT AGG ATG ATA AAG AAA TTT GAC AGA AAA	780
241	Leu His Met Ile Asn Ala His Ala Leu Ala Tyr Arg Met Ile Lys Lys Phe Asp Arg Lys	260
781	AAA GCT GAT CCA GAA TCA AAA GAA CCA GCT GAA ATA GGA ATT ATA TAC AAT AAC ATC GGC	840
261	Lys Ala Asp Pro Glu Ser Lys Glu Pro Ala Glu Ile Gly Ile Ile Tyr Asn Asn Ile Gly	280
841	GTC ACA TAT CCG TTT AAT CCG AAA GAC TCA AAG GAT CTA CAA GCA TCC GAT AAT GCC AAT	900
281	Val Thr Tyr Pro Phe Asn Pro Lys Asp Ser Lys Asp Leu Gln Ala Ser Asp Asn Ala Asn	300
901	TTC TTC CAC AGT GCG CTA TTC TTA ACG GCT ATC CAC AGG GCA AAA TTA AAT ATC GAA TTT	960
301	Phe Phe His Ser Gly Leu Phe Leu Thr Ala Ile His Arg Gly Lys Leu Asn Ile Glu Phe	320
961	GAC GGA GAG ACA TTT GTT TAC CTT CCA TAT TTA AAG GGC AAT GAT TGG CTG GGA GTC AAT	1020
321	Asp Gly Glu Thr Phe Val Tyr Leu Pro Tyr Leu Lys Gly Asn Asp Trp Leu Gly Val Asn	340
1021	TAT TAT ACA AGA GAA GTC GTT AAA TAC CAA GAT CCC ATG TTT CCA AGT ATC CCT CTC ATA	1080
341	Tyr Tyr Thr Arg Glu Val Val Lys Tyr Gln Asp Pro Met Phe Pro Ser Ile Pro Leu Ile	360
1081	AGC TTC AAG GGC GTT CCA GAT TAT GGA TAC GGA TGT AGA CCA GGA ACG ACG TCA AAG GAC	1140
361	Ser Phe Lys Gly Val Pro Asp Tyr Gly Tyr Gly Cys Arg Pro Gly Thr Thr Ser Lys Asp	380
1141	GGT AAT CCT GTT AGT GAC ATT GGA TGG GAG GTA TAT CCC AAA GGC ATG TAC GAC TCT ATA	1200
381	Gly Asn Pro Val Ser Asp Ile Gly Trp Glu Val Tyr Pro Lys Gly Met Tyr Asp Ser Ile	400
1201	GTA GCT CCC AAT GAA TAT GGA GTT CCT GTA TAC GTA ACA GAA AAC GGA ATA GCA GAT TCA	1260
401	Val Ala Ala Asn Glu Tyr Gly Val Pro Val Tyr Val Thr Glu Asn Gly Ile Ala Asp Ser	420
1261	AAA GAT GTA TTA AGC CCC TAT TAC ATC GCA TCT CAC ATT GAA GCC ATG GAA GAG GCT TAC	1320
421	Lys Asp Val Leu Arg Pro Tyr Tyr Ile Ala Ser His Ile Glu Ala Met Glu Glu Ala Tyr	440

Figure 7a

1121	CAA AAT GGT TAT CAC CTC AGA GGA TAC TTA CAC TGG GCA TTA ACC GAT AAT TAC GAA TGG	1180
441	Glu Asn Gly Tyr Asp Val Arg Gly Tyr Leu His Trp Ala Leu Thr Asp Asn Tyr Glu Trp	460
1381	CCC TTA GGG TTC AGA ATG AGG TTT GCC TTC TAC GAA GTA AAC TTG ATA ACC AAA GAG ACA	1440
461	Ala Leu Gly Phe Arg Met Arg Phe Gly Leu Tyr Glu Val Asn Leu Ile Thr Lys Glu Arg	480
1441	AAA CCC AGG AAA AAG AGT GTA AGA GTA TTC AGA GAG ATA GTT ATT AAT AAT GCG CTA ACA	1500
481	Lys Pro Arg Lys Lys Ser Val Arg Val Phe Arg Glu Ile Val Ile Asn Asn Gly Leu Thr	500
1501	AGC AAC ATC AGG AAA CAG ATC TTA CAG CAG GGG TAG	1536
501	Ser Asn Ile Arg Lys Glu Ile Leu Glu Glu Gly End	512

Figure 7b(Continued)

PYROCOCCUS FURIOSUS GLYCOSIDASE - 701  
COMPLETE GENE SEQUENCE - 10/95

1	ATG TTC CTT GAA AAG TTC CTT TGG GGT GTG GCA CAA TCG GGT TTT CAG TTT GAA ATG GGG	60
1	Met Phe Pro Glu Lys Phe Leu Trp Gly Val Ala Gln Ser Gly Phe Gln Phe Glu Met Gly	20
61	GAT AAA CTC AGG AGG AAT ATT GAC ACT AAC ACT GAT TGG TGG CAC TCG CTA AGG GAT AAG	120
21	Asp Lys Leu Arg Arg Asn Ile Asp Thr Asn Thr Asp Trp Trp His Trp Val Arg Asp Lys	40
121	ACA AAT ATA GAG AAA GGC CTC GTT AGT GCA GAT CTT CCC GAG GAG GGG ATT AAC AAT TAC	180
41	Thr Asn Ile Glu Lys Gly Leu Val Ser Gly Asp Leu Pro Glu Glu Gly Ile Asn Asn Tyr	60
181	GAG CTT TAT GAG AAG GAC CAT GAG ATT CCA AGA AAG CTG GGT CTT AAT GCT TAC AGA ATA	240
61	Glu Leu Tyr Glu Lys Asp His Glu Ile Ala Arg Lys Leu Gly Leu Asn Ala Tyr Arg Ile	80
241	GGC ATA GAG TGG AGC AGA ATA TTC CCA TGG CCA ACG ACA TTT ATT GAT GTT GAT TAT AGC	300
81	Gly Ile Glu Trp Ser Arg Ile Phe Pro Trp Pro Thr Thr Phe Ile Asp Val Asp Tyr Ser	100
301	TAT AAT GAA TCA TAT AAC CTT ATA GAA GAT GTA AAG ATC ACC AAG GAC ACT TTG GAG GAG	360
101	Tyr Asn Glu Ser Tyr Asn Leu Ile Glu Asp Val Lys Ile Thr Lys Asp Thr Leu Glu Glu	120
361	TTA GAT GAG ATC GCC AAC AAG AGG GAG GTG GCC TAC TAT AGG TCA GTC ATA AAC AGC CTC	420
121	Leu Asp Glu Ile Ala Asn Lys Arg Glu Val Ala Tyr Tyr Arg Ser Val Ile Asn Ser Leu	140
421	AGG AGC AAG GGG TTT AAG GTT ATA GTT AAT CTA AAT CAC TTC ACC CTT CCA TAT TGG TTG	480
141	Arg Ser Lys Gly Phe Lys Val Ile Val Asn Leu Asn His Phe Thr Leu Pro Tyr Trp Leu	160
481	CAT GAT CCC ATT GAG GCT AGG GAG AGG GCG TTA ACT AAT AAG AGG AAC GGC TGG GTT AAC	540
161	His Asp Pro Ile Glu Ala Arg Glu Arg Ala Leu Thr Asn Lys Arg Asn Gly Trp Val Asn	180
541	CCA AGA ACA GTT ATA GAG TTT GCA AAG TAT GCC GCT TAC ATA GCC TAT AAG TTT GCA GAT	600
191	Pro Arg Thr Val Ile Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala Tyr Lys Phe Gly Asp	200
601	ATA GTG GAT ATG TGG AGC ACG TTT AAT GAG CCT ATG GTG GTT GTT GAG CTT GGC TAC CTA	660
201	Ile Val Asp Met Trp Ser Thr Phe Asn Glu Pro Met Val Val Val Glu Leu Gly Tyr Leu	220
661	GCC CCC TAC TCT GGC TTC CCT CCA GGG GTT CTA AAT CCA GAG GCC CCA AAG CTG GCG ATA	720
221	Ala Pro Tyr Ser Gly Phe Pro Pro Gly Val Leu Asn Pro Glu Ala Ala Lys Leu Ala Ile	240
721	CTT CAC ATG ATA AAT GCA CAT GCT TTA GCT TAT AGG CAG ATA AAG AAG TTT GAC ACT GAG	780
241	Leu His Met Ile Asn Ala His Ala Leu Ala Tyr Arg Gln Ile Lys Lys Phe Asp Thr Glu	260
781	AAA GCT GAT AAG GAT TCT AAA GAG CCT GCA GAA GTT GGT ATA ATT TAC AAC AAC ATT GGA	840
261	Lys Ala Asp Lys Asp Ser Lys Glu Pro Ala Glu Val Gly Ile Ile Tyr Asn Asn Ile Gly	280
841	GTT GCT TAT CCC AAG GAT CCG AAC GAT TCC AAG GAT GTT AAG GCA GCA GAA AAC GAC AAC	900
281	Val Ala Tyr Pro Lys Asp Pro Asn Asp Ser Lys Asp Val Lys Ala Ala Glu Asn Asp Asn	300
901	TTC TTC CAC TCA GGG CTG TTC TTC GAG GCC ATA CAC AAA GGA AAA CTT AAT ATA GAG TTT	960
301	Phe Phe His Ser Gly Leu Phe Phe Glu Ala Ile His Lys Gly Lys Leu Asn Ile Glu Phe	320
961	GAC GGT GAA ACG TTT ATA GAT GCC CCC TAT CTA AAG GGC AAT GAC TGG ATA GGG GTT AAT	1020
321	Asp Gly Glu Thr Phe Ile Asp Ala Pro Tyr Leu Lys Gly Asn Asp Trp Ile Gly Val Asn	340
1021	TAC TAC ACA AGG GAA GTA GTT ACG TAT CAG GAA CCA ATG TTT CCT TCA ATC CCG CTG ATC	1080
341	Tyr Tyr Thr Arg Glu Val Val Thr Tyr Gln Glu Pro Met Phe Pro Ser Ile Pro Leu Ile	360
1081	ACC TTT AAG GGA GTT CAA GCA TAT GGC TAT GCC TGC AGA CCT GGA ACT CTG TCA AAG GAT	1140
361	Thr Phe Lys Gly Val Gln Gly Tyr Gly Tyr Ala Cys Arg Pro Gly Thr Leu Ser Lys Asp	380
1141	GAC AGA CCC GTC AGC GAC ATA GGA TGG GAA CTC TAT CCA GAG GGG ATG TAC GAT TCA ATA	1200
381	Asp Arg Pro Val Ser Asp Ile Gly Trp Glu Leu Tyr Pro Glu Gly Met Tyr Asp Ser Ile	400
1201	GTT CAA GCT CAC AAG TAC GGC GTT CCA GTT TAC GTG ACG GAG AAC GGA ATA GCG GAT TCA	1260
401	Val Glu Ala His Lys Tyr Gly Val Pro Val Tyr Val Thr Glu Asn Gly Ile Ala Asp Ser	420

Figure 8a.



1261	AAG GAC ATC CTA AGA CCT TAC TAC ATA GCG AGC CAC ATA AAG ATG ATA CAG AAG GCC TTT	1320
421	Lys Asp Ile Leu Arg Pro Tyr Tyr Ile Ala Ser His Ile Lys Met Ile Glu Lys Ala Phe	440
1321	GAG GAT GCG TAT GAA GTT AAG GGC TAC TTC CAC TGG GCA TTA ACT GAC AAC TTC GAG TGG	1380
441	Glu Asp Gly Tyr Glu Val Lys Gly Tyr Phe His Trp Ala Leu Thr Asp Asn Phe Glu Trp	460
1381	GCT CTC GCG TTT AGA ATG CCG TTT GCG CTC TAC GAA GTC AAC CTA ATT ACA AAG GAG AGA	1440
461	Ala Leu Gly Phe Arg Met Arg Phe Gly Leu Tyr Glu Val Asn Leu Ile Thr Lys Glu Arg	480
1441	ATT CCC AGG GAG AAG AGC GTG TCG ATA TTC AGA GAG ATA GTA GCC AAT AAT GGT GTT ACG	1500
481	Ile Pro Arg Glu Lys Ser Val Ser Ile Phe Arg Glu Ile Val Ala Asn Asn Gly Val Thr	500
1501	AAA AAG ATT GAA GAG GAA TTG CTG AGG GGA TGA	1533
501	Lys Lys Ile Glu Glu Glu Leu Leu Arg Gly End	511

Figure 8b(Continued)

*Bankia gouldi* endoglucanase (370F1)

9            18            27            36            45            54  
 5' ATG AGA ATA CGT TTA GCG ACG CTC GCG CTC TGC GCA GCG CTG AGC CCA CTC ACC  
    Met Arg Ile Arg Leu Ala Thr Leu Ala Leu Cys Ala Ala Leu Ser Pro Val Thr  
           63            72            81            90            99            108  
    TTT GCA GAT AAT GTA ACC GTA CAA ATC GAC GCC GAC GCC GGT AAA AAA CTC ATC  
    Phe Ala Asp Asn Val Thr Val Gln Ile Asp Ala Asp Gly Gly Lys Lys Leu Ile  
           117            126            135            144            153            162  
    AGC CGA GCC CTT TAC GGC ATG AAT AAC TCC AAC GCA GAA AGC CTT ACC GAT ACT  
    Ser Arg Ala Leu Tyr Gly Met Asn Asn Ser Asn Ala Glu Ser Leu Thr Asp Thr  
           171            180            189            198            207            216  
    GAC TGG CAG CGT TTT CGC GAT GCA GGT GTG CGC ATG CTG CGG GAA AAT GGC GGC  
    Asp Trp Gln Arg Phe Arg Asp Ala Gly Val Arg Met Leu Arg Glu Asn Gly Gly  
           225            234            243            252            261            270  
    AAC AAC AGC ACC AAA TAT AAC TGG CAA CTG CAC CTG AGC AGT CAT CCG GAT TGG  
    Asn Asn Ser Thr Lys Tyr Asn Trp Gln Leu His Leu Ser Ser His Pro Asp Trp  
           279            288            297            306            315            324  
    TAC AAC AAT GTC TAC GCC GGC AAC AAC TGG GAC AAC CGG GTA GCC CTG ATT  
    Tyr Asn Asn Val Tyr Ala Gly Asn Asn Asn Trp Asp Asn Arg Val Ala Leu Ile  
           333            342            351            360            369            378  
    CAG GAA AAC CTG CCC GGC GCC GAC ACC ATG TGG CCA TTC CAG CTC ATC GGT AAG  
    Gln Glu Asn Leu Pro Gly Ala Asp Thr Met Trp Ala Phe Gln Leu Ile Gly Lys  
           387            396            405            414            423            432  
    GTC GCG GCG ACT TCT GCC TAC AAC TTT AAC GAT TGG GAA TTC AAC CAG TCG CAA  
    Val Ala Ala Thr Ser Ala Tyr Asn Phe Asn Asp Trp Glu Phe Asn Gln Ser Gln  
           441            450            459            468            477            486  
    TGG TGG ACC GGC GTC GCT CAG AAT CTC GCT GGC GGC GGT GAA CCC AAT CTG GAC  
    Trp Trp Thr Gly Val Ala Gln Asn Leu Ala Gly Gly Gly Glu Pro Asn Leu Asp  
           495            504            513            522            531            540  
    GGC GGC GGC GAA GCG CTG GTT GAA GGA GAC CCC AAT CTC TAC CTC ATG GAT TGG  
    Gly Gly Gly Glu Ala Leu Val Glu Gly Asp Pro Asn Leu Tyr Leu Met Asp Trp  
           549            558            567            576            585            594  
    TCG CCA GCC GAC ACT GTG GGT ATT CTC GAC CAC TGG TTT GGC GTA AAC GCG CTC  
    Ser Pro Ala Asp Thr Val Gly Ile Leu Asp His Trp Phe Gly Val Asn Gly Leu  
           603            612            621            630            639            648  
    GGC GTG CCG CGT GCG AAA GCC AAA TAC TGG AGT ATG GAT AAC GAG CCC GGC ATC  
    Gly Val Arg Arg Gly Lys Ala Lys Tyr Trp Ser Met Asp Asn Glu Pro Gly Ile  
           657            666            675            684            693            702  
    TGG GTT GCG ACC CAC GAC GAT GTA GTG AAA GAA CAA ACC CCG GTA GAA GAT TTC  
    Trp Val Gly Thr His Asp Asp Val Val Lys Glu Gln Thr Pro Val Glu Asp Phe

Figure 9a

*Bankia* *gouldi* endoglucanase (37071) (continued)

711 720 729 738 747 756  
 CTG CAC ACC TAT TTC GAA ACC GCC AAA AAA GCC CGC GCC AAA TTT CCC GGT ATT  
 Leu His Thr Tyr Phe Glu Thr Ala Lys Lys Ala Arg Ala Lys Phe Pro Gly Ile

765 774 783 792 801 810  
 AAA ATC ACC GGT CCG CTG CCC GCT AAT GAG TGG CAG TGG TAT GCC TGG GCC GGT  
 Lys Ile Thr Gly Pro Val Pro Ala Asn Glu Trp Gln Trp Tyr Ala Trp Gly Gly

819 828 837 846 855 864  
 TTC TCG GTA CCC CAG GAA CAA GGG TTT ATG AGC TGG ATG GAG TAT TTC ATC AAG  
 Phe Ser Val Pro Gln Glu Gln Gly Phe Met Ser Trp Met Glu Tyr Phe Ile Lys

873 882 891 900 909 918  
 CCG GTG TCT GAA GAG CAA CGC CCA AGT GGT GTT CGC CTC CTC GAT GTA CTC GAT  
 Arg Val Ser Glu Glu Gln Arg Ala Ser Gly Val Arg Leu Leu Asp Val Leu Asp

927 936 945 954 963 972  
 CTG CAC TAC TAC CCC GGC GCT TAC AAT GCG GAA GAT ATC GTG CAA TTA CAT CGC  
 Leu His Tyr Tyr Pro Gly Ala Tyr Asn Ala Glu Asp Ile Val Gln Leu His Arg

981 990 999 1008 1017 1026  
 ACG TTC TTC GAC CGC GAC TTT GTT TCA CTG GAT GCC AAC GGG GTG AAA ATG GTA  
 Thr Phe Phe Asp Arg Asp Phe Val Ser Leu Asp Ala Asn Gly Val Lys Met Val

1035 1044 1053 1062 1071 1080  
 GAA GGT GGC TGG GAT GAC AGC ATC AAC AAG GAA TAT ATT TTC GGG CGA GTG AAC  
 Glu Gly Gly Trp Asp Asp Ser Ile Asn Lys Glu Tyr Ile Phe Gly Arg Val Asn

1089 1098 1107 1116 1125 1134  
 GAT TGG CTC GAG GAA TAT ATG GGG CCA GAC CAT GGT GTA ACC CTG GGC TTA ACC  
 Asp Trp Leu Glu Glu Tyr Met Gly Pro Asp His Gly Val Thr Leu Gly Leu Thr

1143 1152 1161 1170 1179 1188  
 GAA ATG TGC GTG CGC AAT GTG AAT CCG ATG ACT ACC GCC ATC TGG TAT GCC TCC  
 Glu Met Cys Val Arg Asn Val Asn Pro Met Thr Thr Ala Ile Trp Tyr Ala Ser

1197 1206 1215 1224 1233 1242  
 ATG CTC GGC ACC TTC GCG GAT AAC GGC GTC GAA ATA TTC ACC CCA TGG TGC TGG  
 Met Leu Gly Thr Phe Ala Asp Asn Gly Val Glu Ile Phe Thr Pro Trp Cys Trp

1251 1260 1269 1278 1287 1296  
 AAC ACC GGA ATG TGG GAA ACA CTC CAC CTC TTC AGC CGC TAC AAC AAA CCT TAT  
 Asn Thr Gly Met Trp Glu Thr Leu His Leu Phe Ser Arg Tyr Asn Lys Pro Tyr

1305 1314 1323 1332 1341 1350  
 CGG GTC GCC TCC AGC TCC AGT CTT GAA GAG TTT GTC AGC GCC TAC AGC TCC ATT  
 Arg Val Ala Ser Ser Ser Ser Leu Glu Glu Phe Val Ser Ala Tyr Ser Ser Ile

1359 1368 1377 1386 1395 1404  
 AAC GAA GCA GAA GAC GCC ATG ACG GTA CTT CTG GTG AAT CGT TCC ACT AGC GAG  
 Asn Glu Ala Glu Asp Ala Met Thr Val Leu Leu Val Asn Arg Ser Thr Ser Glu

Figure 9b(Continued)

*Bankia gouldi* endoglucanase (J7071) (continued)

```

1413      1422      1431      1440      1449      1458
ACC CAC ACC GCC ACT GTC GCT ATC GAC GAT TTC CCA CTG GAT GGC CCC TAC CGC
Thr His Thr Ala Thr Val Ala Ile Asp Asp Phe Pro Leu Asp Gly Pro Tyr Arg

1467      1476      1485      1494      1503      1512
ACC CTG CGC TTA CAC AAC CTG CCG GGG GAG GAA ACC TTC GTA TCT CAC CGA GAC
Thr Leu Arg Leu His Asn Leu Pro Gly Glu Glu Thr Phe Val Ser His Arg Asp

1521      1530      1539      1548      1557      1566
AAC GCC CTG GAA AAA GGT ACA GTG CGC GCC AGC GAC AAT ACG GTA ACA CTC GAG
Asn Ala Leu Glu Lys Gly Thr Val Arg Ala Ser Asp Asn Thr Val Thr Leu Glu

1575      1584      1593      1602      1611
TTG CCC CCT CTG TCC GTT ACT GCA ATA TTG CTC AAG GCC CGG CCC TAA 3'
Leu Pro Pro Leu Ser Val Thr Ala Ile Leu Leu Lys Ala Arg Pro ***

```

Figure 9A (Continued)

*Thalassidroma maritima* Alpha-galactosidase  
Complete Gene Sequence (1045)

```

5'  CTC ATC TCT GTG GAA ATA TTC GGA AAC ACC TTC AGA GAG GGA AGA TTC GTT CTC
    Val Ile Cys Val Glu Ile Phe Gly Lys Thr Phe Arg Glu Gly Arg Phe Val Leu

    63      72      81      90      99      108
    AAA GAG AAA AAC TTC ACA CTT GAG TTC GCG GTG GAG AAG ATA CAC CTT GCG TCG
    Lys Glu Lys Asn Phe Thr Val Glu Phe Ala Val Glu Lys Ile His Leu Gly Trp

    117     126     135     144     153     162
    AAG ATC TCC GGC AGG GTG AAG GCA AGT CCG GGA AGC CTT GAG GTT CTT CGA ACC
    Lys Ile Ser Gly Arg Val Lys Gly Ser Pro Gly Arg Leu Glu Val Leu Arg Thr

    171     180     189     198     207     216
    AAA GCA CCG GAA AAG GTA CTT GTG AAC AAC TGG CAG TCC TGG GGA CCG TGC AGG
    Lys Ala Pro Glu Lys Val Leu Val Asn Asn Trp Gln Ser Trp Gly Pro Cys Arg

    225     234     243     252     261     270
    GTG GTC GAT GCC TTT TCT TTC AAA CCA CCT GAA ATA GAT CCG AAC TGG AGA TAC
    Val Val Asp Ala Phe Ser Phe Lys Pro Pro Glu Ile Asp Pro Asn Trp Arg Tyr

    279     288     297     306     315     324
    ACC GCT TCG GTG GTG CCC GAT GEA CTT GAA AGG AAC CTC CAG AGC GAC TAT TTC
    Thr Ala Ser Val Val Pro Asp Val Leu Glu Arg Asn Leu Gln Ser Asp Tyr Phe

    333     342     351     360     369     378
    GTG GCT GAA GAA GGA AAA GTG TAC GGT TTT CTG AGT TCG AAA ATC GCA CAT CCT
    Val Ala Glu Glu Gly Lys Val Tyr Gly Phe Leu Ser Ser Lys Ile Ala His Pro

    387     396     405     414     423     432
    TTC TTC GCT GTG GAA GAT GCG GAA CTT GTG GCA TAC CTC GAA TAT TTC GAT GTC
    Phe Phe Ala Val Glu Asp Gly Glu Leu Val Ala Tyr Leu Glu Tyr Phe Asp Val

    441     450     459     468     477     486
    GAG TTC GAC GAC TTT GTT CCT CTT GAA CCT CTC GTT GTA CTC GAG GAT CCC AAC
    Glu Phe Asp Asp Phe Val Pro Leu Glu Pro Leu Val Val Leu Glu Asp Pro Asn

    495     504     513     522     531     540
    ACA CCC CTT CTT CTG GAG AAA TAC GCG GAA CTC GTC GGA ATG GAA AAC AAC GCG
    Thr Pro Leu Leu Leu Glu Lys Tyr Ala Glu Leu Val Gly Met Glu Asn Asn Ala

    549     558     567     576     585     594
    AGA GTT CCA AAA CAC ACA CCC ACT CCA TCG TGC AGC TCG TAC CAT TAC TTC CTT
    Arg Val Pro Lys His Thr Pro Thr Gly Trp Cys Ser Trp Tyr His Tyr Phe Leu

```

Figure 10a

*Thermotoga maritima* Alpha-galactosidase  
Complete Gene Sequence (2 of 3)

```

      603      612      621      630      639      648
GAT CTC ACC TCG GAA CAG ACT CTC AAG AAC CTC AAG CTC CCG AAG AAT TTC CCG
-----
Asp Leu Thr Trp Glu Glu Thr Leu Lys Asn Leu Lys Leu Ala Lys Asn Phe Pro

      657      666      675      684      693      702
TTC GAG GTC TTC CAG ATA GAC GAC GCG TAC GAA AAG CAC ATA GGT GAC TGG CTC
-----
Phe Glu Val Phe Gln Ile Asp Asp Ala Tyr Glu Lys Asp Ile Gly Asp Trp Leu

      711      720      729      738      747      756
GTG ACA AGA GGA GAC TTT CCA TCG GTG GAA GAG ATG GCA AAA GTT ATA CCG GAA
-----
Val Thr Arg Gly Asp Phe Pro Ser Val Glu Glu Met Ala Lys Val Ile Ala Glu

      765      774      783      792      801      810
AAC GGT TTC ATC CCG GGC ATA TGG ACC GCG CCG TTC AGT GTT TCT GAA ACC TCC
-----
Asn Gly Phe Ile Pro Gly Ile Trp Thr Ala Pro Phe Ser Val Ser Glu Thr Ser

      819      828      837      846      855      864
GAT GTA TTC AAC GAA CAT CCG GAC TGG GTA GTG AAG GAA AAC GGA GAG CCG AAG
-----
Asp Val Phe Asn Glu His Pro Asp Trp Val Val Lys Glu Asn Gly Glu Pro Lys

      873      882      891      900      909      918
ATG GCT TAC AGA AAC TCG AAC AAA AAG ATA TAC GCC CTC GAT CTT TCG AAA GAT
-----
Met Ala Tyr Arg Asn Trp Asn Lys Lys Ile Tyr Ala Leu Asp Leu Ser Lys Asp

      927      936      945      954      963      972
GAG GTT CTG AAC TCG CTT TTC GAT CTC TTC TCA TCT CTG AGA AAG ATG GCG TAC
-----
Glu Val Leu Asn Trp Leu Phe Asp Leu Phe Ser Ser Leu Arg Lys Met Gly Tyr

      981      990      999      1008      1017      1026
AGG TAC TTC AAG ATC GAC TTT CTC TTC CCG GGT GCC GTT CCA GGA GAA AGA AAA
-----
Arg Tyr Phe Lys Ile Asp Phe Leu Phe Ala Gly Ala Val Pro Gly Glu Arg Lys

      1035      1044      1053      1062      1071      1080
AAG AAC ATA ACA CCA ATT CAG CCG TTC AGA AAA GCG ATT GAG ACG ATC AGA AAA
-----
Lys Asn Ile Thr Pro Ile Gln Ala Phe Arg Lys Gly Ile Glu Thr Ile Arg Lys

      1089      1098      1107      1116      1125      1134
GCC GTG GGA GAA GAT TCT TTC ATC CTC GGA TGC GCG TCT CCC CTT CTT CCC GCA
-----
Ala Val Gly Glu Asp Ser Phe Ile Leu Gly Cys Gly Ser Pro Leu Leu Pro Ala

      1143      1152      1161      1170      1179      1188
CTG GGA TGC CTC GAC GCG ATG AGG ATA GGA CCT GAC ACT GCG CCG TTC TGG GGA
-----
Val Gly Cys Val Asp Gly Met Arg Ile Gly Pro Asp Thr Ala Pro Phe Trp Gly

```

Figure 10 (Continued)

*Thermotoga maritima* Alpha-galactosidase  
Complete Gene Sequence (3014)

```

1197      1206      1215      1224      1233      1242
GAA CAT ATA GAA GAC AAC GAA GCT CTC GCT GCA AGA TCG GCG CTG AGA AAC GCC
---
Glu His Ile Glu Asp Asn Gly Ala Pro Ala Ala Arg Trp Ala Leu Arg Asn Ala

1251      1260      1269      1278      1287      1296
ATA ACG AGG TAC TTC ATG CAC GAC ACG TTC TCG CTG AAC GAC CCC GAC TGT CTG
---
Ile Thr Arg Tyr Phe Met His Asp Arg Phe Trp Leu Asn Asp Pro Asp Cys Leu

1305      1314      1323      1332      1341      1350
ATA CTG AGA GAG GAG AAA ACG GAT CTC ACA CAG AAG GAA AAG GAG CTC TAC TCG
---
Ile Leu Arg Glu Glu Lys Thr Asp Leu Thr Gln Lys Glu Lys Glu Leu Tyr Ser

1359      1368      1377      1386      1395      1404
TAC ACG TGT GGA GTG CTC GAC AAC ATG ATC ATA GAA AGC GAT GAT CTC TCG CTC
---
Tyr Thr Cys Gly Val Leu Asp Asn Met Ile Ile Glu Ser Asp Asp Leu Ser Leu

1413      1422      1431      1440      1449      1458
GTC AGA GAT CAT GGA AAA AAG GTT CTG AAA GAA ACG CTC GAA CTC CTC GGT GGA
---
Val Arg Asp His Gly Lys Lys Val Leu Lys Glu Thr Leu Glu Leu Leu Gly Gly

1467      1476      1485      1494      1503      1512
AGA CCA CCG GTT CAA AAC ATC ATG TCG GAG GAT CTG AGA TAC GAG ATC GTC TCG
---
Arg Pro Arg Val Gln Asn Ile Met Ser Glu Asp Leu Arg Tyr Glu Ile Val Ser

1521      1530      1539      1548      1557      1566
TCT GGC ACT CTC TCA CCA AAC GTC AAG ATC GTG GTC GAT CTG AAC AGC AGA GAG
---
Ser Gly Thr Leu Ser Gly Asn Val Lys Ile Val Val Asp Leu Asn Cys Arg Glu

1575      1584      1593      1602      1611      1620
TAC CAC CTG GAA AAA GAA GGA AAG TCC TCC CTG AAA AAA AGA GTC GTC AAA AGA
---
Tyr His Leu Glu Lys Glu Gly Lys Ser Ser Leu Lys Lys Arg Val Val Lys Arg

1629      1638      1647      1656      1665
GAA GAC GGA AGA AAC TTC TAC TTC TAC GAA GAG GGT GAG AGA GAA TGA 3'
---
Glu Asp Gly Arg Asn Phe Tyr Phe Tyr Glu Glu Gly Glu Arg Glu ***

```

Figure 10c(Continued)

*Thermotoga maritima*  $\beta$ -mannanase (~~6699~~) (GGP2)

```

5'   9       18       27       36       45       54
    ATG GGG ATT GGT GGC GAC GAC TCC TGG AGC CCG TCA GTA TCG GCG GAA TTC CTT
    ---
    Met Gly Ile Gly Gly Asp Asp Ser Trp Ser Pro Ser Val Ser Ala Glu Phe Leu

       63       72       81       90       99       108
    TTA TTG ATC GTT GAG CTC TCT TTC GTT CTC TTT GCA AGT GAC GAG TTC GTG AAA
    ---
    Leu Leu Ile Val Glu Leu Ser Phe Val Leu Phe Ala Ser Asp Glu Phe Val Lys

       117       126       135       144       153       162
    GTG GAA AAC GGA AAA TTC GCT CTG AAC GGA AAA GAA TTC AGA TTC ATT GGA AGC
    ---
    Val Glu Asn Gly Lys Phe Ala Leu Asn Gly Lys Glu Phe Arg Phe Ile Gly Ser

       171       180       189       198       207       216
    AAC AAC TAC TAC ATG CAC TAC AAG AGC AAC GGA ATG ATA GAC AGT GTT CTG GAG
    ---
    Asn Asn Tyr Tyr Met His Tyr Lys Ser Asn Gly Met Ile Asp Ser Val Leu Glu

       225       234       243       252       261       270
    AGT GCC AGA GAC ATG GGT ATA AAG GTC CTC AGA ATC TGG GGT TTC CTC GAC GGG
    ---
    Ser Ala Arg Asp Met Gly Ile Lys Val Leu Arg Ile Trp Gly Phe Leu Asp Gly

       279       288       297       306       315       324
    GAG AGT TAC TGC AGA GAC AAG AAC ACC TAC ATG CAT CCT GAG CCC GGT GTT TTC
    ---
    Glu Ser Tyr Cys Arg Asp Lys Asn Thr Tyr Met His Pro Glu Pro Gly Val Phe

       333       342       351       360       369       378
    GGG GTG CCA GAA GGA ATA TCG AAC GCC CAG AGC GGT TTC GAA AGA CTC GAC TAC
    ---
    Gly Val Pro Glu Gly Ile Ser Asn Ala Gln Ser Gly Phe Glu Arg Leu Asp Tyr

       387       396       405       414       423       432
    ACA GTT GCG AAA GCG AAA GAA CTC GGT ATA AAA CTT GTC ATT GTT CTT GTG AAC
    ---
    Thr Val Ala Lys Ala Lys Glu Leu Gly Ile Lys Leu Val Ile Val Leu Val Asn

       441       450       459       468       477       486
    AAC TGG GAC GAC TTC GGT GGA ATG AAC CAG TAC GTG AGG TGG TTT GGA GGA ACC
    ---
    Asn Trp Asp Asp Phe Gly Gly Met Asn Gln Tyr Val Arg Trp Phe Gly Gly Thr

       495       504       513       522       531       540
    CAT CAC GAC GAT TTC TAC AGA GAT GAG AAG ATC AAA GAA GAG TAC AAA AAG TAC
    ---
    His His Asp Asp Phe Tyr Arg Asp Glu Lys Ile Lys Glu Glu Tyr Lys Lys Tyr
  
```

Figure 11a



*Thermotoga maritima*  $\beta$ -mannanase (22623) (continued) (6612)

549	558	567	576	585	594
GTC TCC TTT CTC GTA AAC CAT GTC AAT ACC TAC ACG GGA GTT CCT TAC AGG GAA					
Val Ser Phe Leu Val Asn His Val Asn Thr Tyr Thr Gly Val Pro Tyr Arg Glu					
603	612	621	630	639	648
GAG CCC ACC ATC ATG GCC TGG GAG CTT GCA AAC GAA CCG CCC TGT GAG ACG GAC					
Glu Pro Thr Ile Met Ala Trp Glu Leu Ala Asn Glu Pro Arg Cys Glu Thr Asp					
657	666	675	684	693	702
AAA TCG GGG AAC ACG CTC GTT GAG TGG GTG AAG GAG ATG AGC TCC TAC ATA AAG					
Lys Ser Gly Asn Thr Leu Val Glu Trp Val Lys Glu Met Ser Ser Tyr Ile Lys					
711	720	729	738	747	756
AGT CTG GAT CCC AAC CAC CTC GTG GCT GTG GCG GAC GAA GGA TTC TTC AGC AAC					
Ser Leu Asp Pro Asn His Leu Val Ala Val Gly Asp Glu Gly Phe Phe Ser Asn					
765	774	783	792	801	810
TAC GAA GGA TTC AAA CCT TAC GGT GGA GAA GCC GAG TGG GCC TAC AAC GCC TGG					
Tyr Glu Gly Phe Lys Pro Tyr Gly Gly Glu Ala Glu Trp Ala Tyr Asn Gly Trp					
819	828	837	846	855	864
TCC GGT GTT GAC TGG AAG AAG CTC CTT TCG ATA GAG ACG GTG GAC TTC GCC ACG					
Ser Gly Val Asp Trp Lys Lys Leu Leu Ser Ile Glu Thr Val Asp Phe Gly Thr					
873	882	891	900	909	918
TTC CAC CTC TAT CCG TCC CAC TGG GGT GTC AGT CCA GAG AAC TAT GCC CAG TGG					
Phe His Leu Tyr Pro Ser His Trp Gly Val Ser Pro Glu Asn Tyr Ala Gln Trp					
927	936	945	954	963	972
GGA GCG AAG TGG ATA GAA GAC CAC ATA AAG ATC GCA AAA GAG ATC GGA AAA CCC					
Gly Ala Lys Trp Ile Glu Asp His Ile Lys Ile Ala Lys Glu Ile Gly Lys Pro					
981	990	999	1008	1017	1026
GTT GTT CTG GAA GAA TAT GGA ATT CCA AAG AGT GCG CCA GTT AAC AGA ACG GCC					
Val Val Leu Glu Glu Tyr Gly Ile Pro Lys Ser Ala Pro Val Asn Arg Thr Ala					
1035	1044	1053	1062	1071	1080
ATC TAC AGA CTC TGG AAC GAT CTG GTC TAC GAT CTC GGT GGA GAT GGA GCG ATG					
Ile Tyr Arg Leu Trp Asn Asp Leu Val Tyr Asp Leu Gly Gly Asp Gly Ala Met					

Figure 11b(Continued)

*Thermotoga maritima*  $\beta$ -mannanase (6627) (continued) (66P2)

1089	1098	1107	1116	1125	1134
TTC TGG ATG CTC GCG GGA ATC GGG GAA GGT TCG GAC AGA GAC GAG AGA CCG TAC					
---	---	---	---	---	---
Phe Trp Met Leu Ala Gly Ile Gly Glu Gly Ser Asp Arg Asp Glu Arg Gly Tyr					
1143	1152	1161	1170	1179	1188
TAT CCG GAC TAC GAC GGT TTC AGA ATA GTG AAC GAC GAC AGT CCA GAA GCG GAA					
---	---	---	---	---	---
Tyr Pro Asp Tyr Asp Gly Phe Arg Ile Val Asn Asp Asp Ser Pro Glu Ala Glu					
1197	1206	1215	1224	1233	1242
CTG ATA AGA GAA TAC GCG AAG CTG TTC AAC ACA GGT GAA GAC ATA AGA GAA GAC					
---	---	---	---	---	---
Leu Ile Arg Glu Tyr Ala Lys Leu Phe Asn Thr Gly Glu Asp Ile Arg Glu Asp					
1251	1260	1269	1278	1287	1296
ACC TGC TCT TTC ATC CTT CCA AAA GAC GGC ATG GAG ATC AAA AAG ACC GTG GAA					
---	---	---	---	---	---
Thr Cys Ser Phe Ile Leu Pro Lys Asp Gly Met Glu Ile Lys Lys Thr Val Glu					
1305	1314	1323	1332	1341	1350
GTG ACG GCT GGT GTT TTC GAC TAC AGC AAC ACG TTT GAA AAG TTG TCT GTC AAA					
---	---	---	---	---	---
Val Arg Ala Gly Val Phe Asp Tyr Ser Asn Thr Phe Glu Lys Leu Ser Val Lys					
1359	1368	1377	1386	1395	1404
GTC GAA GAT CTG GTT TTT GAA AAT GAC ATA GAG CAT CTC GGA TAC GGA ATT TAC					
---	---	---	---	---	---
Val Glu Asp Leu Val Phe Glu Asn Glu Ile Glu His Leu Gly Tyr Gly Ile Tyr					
1413	1422	1431	1440	1449	1458
GGC TTT GAT CTC GAC ACA ACC CCG ATC CCG GAT GGA GAA CAT GAA ATG TTC CTT					
---	---	---	---	---	---
Gly Phe Asp Leu Asp Thr Thr Arg Ile Pro Asp Gly Glu His Glu Met Phe Leu					
1467	1476	1485	1494	1503	1512
GAA GGC CAC TTT CAG GGA AAA ACG GTG AAA GAC TCT ATC AAA GCG AAA GTG GTG					
---	---	---	---	---	---
Glu Gly His Phe Gln Gly Lys Thr Val Lys Asp Ser Ile Lys Ala Lys Val Val					
1521	1530	1539	1548	1557	1566
AAC GAA GCA CCG TAC GTG CTC GCA GAG GAA GTT GAT TTT TCC TCT CCA GAA GAG					
---	---	---	---	---	---
Asn Glu Ala Arg Tyr Val Leu Ala Glu Glu Val Asp Phe Ser Ser Pro Glu Glu					
1575	1584	1593	1602	1611	1620
GTG AAA AAC TGG TGG AAC AGC GGA ACC TGG CAG GAG TTC GGG TCA CCT GAC					
---	---	---	---	---	---
Val Lys Asn Trp Trp Asn Ser Gly Thr Trp Gln Ala Glu Phe Gly Ser Pro Asp					

Figure 11C(Continued)

*Thermotoga maritima*  $\beta$ -mannanase (662) (continued) (66P2)

1629	1638	1647	1656	1665	1674
ATT GAA TGG AAC GGT GAG GTG GGA AAT GGA GCA CTG CAG CTG AAC GTG AAA CTC					
Ile Glu Trp Asn Gly Glu Val Gly Asn Gly Ala Leu Gln Leu Asn Val Lys Leu					
1683	1692	1701	1710	1719	1728
CCC GGA AAG AGC GAC TGG GAA GAA GTG AGA GTA GCA AGG AAG TTC GAA AGA CTC					
Pro Gly Lys Ser Asp Trp Glu Glu Val Arg Val Ala Arg Lys Phe Glu Arg Leu					
1737	1746	1755	1764	1773	1782
TCA GAA TGT GAG ATC CTC GAG TAC GAC ATC TAC ATT CCA AAC GTC GAG GGA CTC					
Ser Glu Cys Glu Ile Leu Glu Tyr Asp Ile Tyr Ile Pro Asn Val Glu Gly Leu					
1791	1800	1809	1818	1827	1836
AAG CGA AGG TTG AGG CCG TAC GCG GTT CTG AAC CCC GGC TGG GTG AAG ATA GGC					
Lys Gly Arg Leu Arg Pro Tyr Ala Val Leu Asn Pro Gly Trp Val Lys Ile Gly					
1845	1854	1863	1872	1881	1890
CTC GAC ATG AAC AAC GCG AAC GTT GAA AGT GCG GAG ATC ATC ACT TTC GGC GGA					
Leu Asp Met Asn Asn Ala Asn Val Glu Ser Ala Glu Ile Ile Thr Phe Gly Gly					
1899	1908	1917	1926	1935	1944
AAA GAG TAC AGA AGA TTC CAT GTA AGA ATT GAG TTC GAC AGA ACA GCG GGG GTG					
Lys Glu Tyr Arg Arg Phe His Val Arg Ile Glu Phe Asp Arg Thr Ala Gly Val					
1953	1962	1971	1980	1989	1998
AAA GAA CTT CAC ATA GGA CTT GTC GGT CAT CAT CTG AGG TAC GAT GGA CCG ATT					
Lys Glu Leu His Ile Gly Val Val Gly Asp His Leu Arg Tyr Asp Gly Pro Ile					
2007	2016	2025	2034	2043	
TTC ATC GAT AAT GTG AGA CTT TAT AAA AGA ACA GGA GGT ATG TGA 3'					
Phe Ile Asp Asn Val Arg Leu Tyr Lys Arg Thr Gly Gly Met ...					

Figure 11d (Continued)

ARPII 1a  $\beta$ -mannosidase (630B1)

```

5'  ATG CTA CCA GAA GAG TTC CTA TGG GGC GTT GCG CAG TCA GGC TTT CAG TTC GAA 54
    ---
Met Leu Pro Glu Glu Phe Leu Trp Gly Val Gly Gln Ser Gly Phe Gln Phe Glu

    63      72      81      90      99      108
ATG GGC GAC AAG CTC AGG AGG CAC ATC GAT CCA AAT ACC GAC TGG TGG AAG TGG
---
Met Gly Asp Lys Leu Arg Arg His Ile Asp Pro Asn Thr Asp Trp Trp Lys Trp

    117     126     135     144     153     162
GTT CGC GAT CCT TTC AAC ATA AAA AAG GAG CTT GTG AGT GCG GAC CTT CCC GAG
---
Val Arg Asp Pro Phe Asn Ile Lys Lys Glu Leu Val Ser Gly Asp Leu Pro Glu

    171     180     189     198     207     216
GAC GGC ATC AAC AAC TAC GAA CTT TTT GAA AAC GAT CAC AAG CTC GCT AAA GGC
---
Asp Gly Ile Asn Asn Tyr Glu Leu Phe Glu Asn Asp His Lys Leu Ala Lys Gly

    225     234     243     252     261     270
CTT GGA CTC AAC GCA TAC AGG ATT GGA ATA GAG TGG ACC AGA ATC TTT CCC TGG
---
Leu Gly Leu Asn Ala Tyr Arg Ile Gly Ile Glu Trp Ser Arg Ile Phe Pro Trp

    279     288     297     306     315     324
CCG ACG TGG ACG GTC GAT ACC GAG GTC GAG TTC GAC ACT TAC GGT TTA GTA AAG
---
Pro Thr Trp Thr Val Asp Thr Glu Val Glu Phe Asp Thr Tyr Gly Leu Val Lys

    333     342     351     360     369     378
GAC GTT AAG ATA GAC AAG TCC ACC CTT GCT GAA CTC GAC AGG CTG GCC AAC AAG
---
Asp Val Lys Ile Asp Lys Ser Thr Leu Ala Glu Leu Asp Arg Leu Ala Asn Lys

    387     396     405     414     423     432
GAG GAG GTA ATG TAC TAC AGG CGC GTT ATT CAG CAT TTG AGG GAG CTC GCC TTC
---
Glu Glu Val Met Tyr Tyr Arg Arg Val Ile Gln His Leu Arg Glu Leu Gly Phe

    441     450     459     468     477     486
AAG GTC TTC GTT AAC CTC AAC CAC TTC ACG CTT CCA ATA TGG CTC CAC GAC CCG
---
Lys Val Phe Val Asn Leu Asn His Phe Thr Leu Pro Ile Trp Leu His Asp Pro

    495     504     513     522     531     540
ATA GTG GCA AGG GAG AAG GCC CTC ACA AAC GAC AGA ATC GGC TGG GTC TCC CAG
---
Ile Val Ala Arg Glu Lys Ala Leu Thr Asn Asp Arg Ile Gly Trp Val Ser Gln

```

Figure 12A.

AEP11 1a  $\beta$ -mannosidase (6J0B1) (continued)

549	558	567	576	585	594
AGG ACA GTT GTT GAG TTT GCC AAG TAT GCT GCT TAC ATC GCC CAT GCG CTC GGA					
Arg Thr Val Val Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala His Ala Leu Gly					
603	612	621	630	639	648
GAC CTC GTG GAC ACA TCG AGC ACC TTC AAC GAA CCT ATG GTA GTT GTG GAG CTC					
Asp Leu Val Asp Thr Trp Ser Thr Phe Asn Glu Pro Met Val Val Val Glu Leu					
657	666	675	684	693	702
GGC TAC CTC GCC CCC TAC TCA GGA TTT CCC CCG GGA GTC ATG AAC CCC GAG GCC					
Gly Tyr Leu Ala Pro Tyr Ser Gly Phe Pro Pro Gly Val Met Asn Pro Glu Ala					
711	720	729	738	747	756
GCG AAG CTG GCG ATC CTC AAC ATG ATA AAC GCC CAC GCC TTG GCA TAT AAG ATG					
Ala Lys Leu Ala Ile Leu Asn Met Ile Asn Ala His Ala Leu Ala Tyr Lys Met					
765	774	783	792	801	810
ATA AAG AGG TTC GAC ACC AAG AAG GCC GAT GAG GAT AGC AAG TCC CCT GCG GAC					
Ile Lys Arg Phe Asp Thr Lys Lys Ala Asp Glu Asp Ser Lys Ser Pro Ala Asp					
819	828	837	846	855	864
GTT GGC ATA ATT TAC AAC AAC ATC GGT GTT GCC TAC CCT AAA GAC CCT AAC GAT					
Val Gly Ile Ile Tyr Asn Asn Ile Gly Val Ala Tyr Pro Lys Asp Pro Asn Asp					
873	882	891	900	909	918
CCC AAG GAC GTT AAA GCA GCC GAA AAC GAC AAC TAC TTC CAC AGC GGA CTG TTC					
Pro Lys Asp Val Lys Ala Ala Glu Asn Asp Asn Tyr Phe His Ser Gly Leu Phe					
927	936	945	954	963	972
TTT GAT GCC ATC CAC AAG GGT AAG CTC AAC ATA GAG TTC GAC GGC GAA AAC TTT					
Phe Asp Ala Ile His Lys Gly Lys Leu Asn Ile Glu Phe Asp Gly Glu Asn Phe					
981	990	999	1008	1017	1026
GTA AAA GTT AGA CAC CTA AAA GGC AAT GAC TGG ATA GGC CTC AAC TAC TAC ACC					
Val Lys Val Arg His Leu Lys Gly Asn Asp Trp Ile Gly Leu Asn Tyr Tyr Thr					
1035	1044	1053	1062	1071	1080
GCG GAG GTT GTT AGA TAT TCG GAG CCC AAG TTC CCA AGT ATA CCC CTC ATA TCC					
Arg Glu Val Val Arg Tyr Ser Glu Pro Lys Phe Pro Ser Ile Pro Leu Ile Ser					

Figure 12b(Continued)

ANP11 1a  $\beta$ -mannosidase (63081) (continued)

```

1089      1098      1107      1116      1125      1134
TTC AAG GGC GTT CCC AAC TAC GGC TAC TCC TGC AGG CCC GGC ACG ACC TCC GCC
-----
Phe Lys Gly Val Pro Asn Tyr Gly Tyr Ser Cys Arg Pro Gly Thr Thr Ser Ala

1143      1152      1161      1170      1179      1188
GAT GGC ATG CCC GTC AGC GAT ATC GGC TGG GAA GTC TAT CCC CAG GGA ATC TAC
-----
Asp Gly Met Pro Val Ser Asp Ile Gly Trp Glu Val Tyr Pro Gln Gly Ile Tyr

1197      1206      1215      1224      1233      1242
GAC TCG ATA GTC GAG GCC ACC AAG TAC AGT GTT CCT GTT TAC GTC ACC GAG AAC
-----
Asp Ser Ile Val Glu Ala Thr Lys Tyr Ser Val Pro Val Tyr Val Thr Glu Asn

1251      1260      1269      1278      1287      1296
GGT GTT GCG GAT TCC GCG GAC ACG CTG AGG CCA TAC TAC ATA GTC AGC CAC GTC
-----
Gly Val Ala Asp Ser Ala Asp Thr Leu Arg Pro Tyr Tyr Ile Val Ser His Val

1305      1314      1323      1332      1341      1350
TCA AAG ATA GAG GAA GCC ATT GAG AAT GGA TAC CCC GTA AAA GGC TAC ATG TAC
-----
Ser Lys Ile Glu Glu Ala Ile Glu Asn Gly Tyr Pro Val Lys Gly Tyr Met Tyr

1359      1368      1377      1386      1395      1404
TGG CCG CTT ACG GAT AAC TAC GAG TGG GCC CTC GGC TTC AGC ATG AGG TTT GGT
-----
Trp Ala Leu Thr Asp Asn Tyr Glu Trp Ala Leu Gly Phe Ser Met Arg Phe Gly

1413      1422      1431      1440      1449      1458
CTC TAC AAG GTC GAC CTC ATC TCC AAG GAG AGG ATC CCG AGG GAG AGA AGC GTT
-----
Leu Tyr Lys Val Asp Leu Ile Ser Lys Glu Arg Ile Pro Arg Glu Arg Ser Val

1467      1476      1485      1494      1503      1512
GAG ATA TAT CCG AGG ATA GTG CAG TCC AAC GGT GTT CCT AAG GAT ATC AAA GAG
-----
Glu Ile Tyr Arg Arg Ile Val Gln Ser Asn Gly Val Pro Lys Asp Ile Lys Glu

1521      1530      1539
GAG TTC CTG AAG GGT GAG GAG AAA TGA 3'
-----
Glu Phe Leu Lys Gly Glu Glu Lys ...

```

Figure 12C(Continued)

## OC1/4V Endoglucanase (33GP1)

```

      9      18      27      36      45      54
5' ATG GTA GAA AGA CAC TTC AGA TAT GTT CTT ATT TGC ACC CTG TTT CTT GTT ATG
   Met Val Glu Arg His Phe Arg Tyr Val Leu Ile Cys Thr Leu Phe Leu Val Met

      63      72      81      90      99      108
   CTC CTA ATC TCA TCC ACT CAG TGT GGA AAA AAT GAA CCA AAC AAA AGA GTG AAT
   Leu Leu Ile Ser Ser Thr Gln Cys Gly Lys Asn Glu Pro Asn Lys Arg Val Asn

      117      126      135      144      153      162
   AGC ATG GAA CAG TCA GTT GCT GAA AGT GAT AGC AAC TCA GCA TTT GAA TAC AAC
   Ser Met Glu Gln Ser Val Ala Glu Ser Asp Ser Asn Ser Ala Phe Glu Tyr Asn

      171      180      189      198      207      216
   AAA ATG GTA GGT AAA GGA GTA AAT ATT GGA AAT GCT TTA GAA GCT CCT TTC GAA
   Lys Met Val Gly Lys Gly Val Asn Ile Gly Asn Ala Leu Glu Ala Pro Phe Glu

      225      234      243      252      261      270
   GGA GCT TGG GGA GTA AGA ATT GAG GAT GAA TAT TTT CAG ATA ATA AAG AAA AGG
   Gly Ala Trp Gly Val Arg Ile Glu Asp Glu Tyr Phe Glu Ile Ile Lys Lys Arg

      279      288      297      306      315      324
   GGA TTT GAT TCT GTT AGG ATT CCC ATA AGA TGG TCA GCA CAT ATA TCC GAA AAG
   Gly Phe Asp Ser Val Arg Ile Pro Ile Arg Trp Ser Ala His Ile Ser Glu Lys

      333      342      351      360      369      378
   CCA CCA TAT GAT ATT GAC AGG AAT TTC CTC GAA AGA GTT AAC CAT GTT GTC GAT
   Pro Pro Tyr Asp Ile Asp Arg Asn Phe Leu Glu Arg Val Asn His Val Val Asp

      387      396      405      414      423      432
   AGG GCT CTT GAG AAT AAT TTA ACA GTA ATC ATC AAT ACG CAC CAT TTT GAA GAA
   Arg Ala Leu Glu Asn Asn Leu Thr Val Ile Ile Asn Thr His His Phe Glu Glu

      441      450      459      468      477      486
   CTC TAT CAA GAA CCG GAT AAA TAC GGC GAT GTT TTG GTG GAA ATT TGG AGA CAG
   Leu Tyr Gln Glu Pro Asp Lys Tyr Gly Asp Val Leu Val Glu Ile Trp Arg Gln

      495      504      513      522      531      540
   ATT GCA AAA TTC TTT AAA GAT TAC CCG GAA AAT CTG TTC TTT GAA ATC TAC AAC
   Ile Ala Lys Phe Phe Lys Asp Tyr Pro Glu Asn Leu Phe Phe Glu Ile Tyr Asn

```

Figure 13A

OC1/4V Endoglucanase (33GF1) (continued)

549	558	567	576	585	594
GAG CCT GCT CAG AAC TTG ACA GCT GAA AAA TGG AAC GCA CTT TAT CCA AAA GTG					
---	---	---	---	---	---
Glu Pro Ala Gln Asn Leu Thr Ala Glu Lys Trp Asn Ala Leu Tyr Pro Lys Val					
603	612	621	630	639	648
CTC AAA GTT ATC AGG GAG AGC AAT CCA ACC CGG ATT GTC ATT ATC GAT GCT CCA					
---	---	---	---	---	---
Leu Lys Val Ile Arg Glu Ser Asn Pro Thr Arg Ile Val Ile Ile Asp Ala Pro					
657	666	675	684	693	702
AAC TGG GCA CAC TAT AGC GCA GTG AGA AGT CTA AAA TTA GTC AAC GAC AAA CGC					
---	---	---	---	---	---
Asn Trp Ala His Tyr Ser Ala Val Arg Ser Leu Lys Leu Val Asn Asp Lys Arg					
711	720	729	738	747	756
ATC ATT GTT TCC TTC CAT TAC TAC GAA CCT TTC AAA TTC ACA CAT CAG GGT GCC					
---	---	---	---	---	---
Ile Ile Val Ser Phe His Tyr Tyr Glu Pro Phe Lys Phe Thr His Gln Gly Ala					
765	774	783	792	801	810
GAA TGG GTT AAT CCC ATC CCA CCT GTT AGG GTT AAG TGG AAT GGC GAC GAA TGG					
---	---	---	---	---	---
Glu Trp Val Asn Pro Ile Pro Pro Val Arg Val Lys Trp Asn Gly Glu Glu Trp					
819	828	837	846	855	864
GAA ATT AAC CAA ATC AGA AGT CAT TTC AAA TAC GTG AGT GAC TGG GCA AAG CAA					
---	---	---	---	---	---
Glu Ile Asn Gln Ile Arg Ser His Phe Lys Tyr Val Ser Asp Trp Ala Lys Gln					
873	882	891	900	909	918
AAT AAC GTA CCA ATC TTT CTT GGT GAA TTC GGT GCT TAT TCA AAA GCA GAC ATG					
---	---	---	---	---	---
Asn Asn Val Pro Ile Phe Leu Gly Glu Phe Gly Ala Tyr Ser Lys Ala Asp Met					
927	936	945	954	963	972
GAC TCA AGG GTT AAG TGG ACC GAA AGT GTG AGA AAA ATG GCG GAA GAA TTT GGA					
---	---	---	---	---	---
Asp Ser Arg Val Lys Trp Thr Glu Ser Val Arg Lys Met Ala Glu Glu Phe Gly					
981	990	999	1008	1017	1026
TTT TCA TAC GCG TAT TGG GAA TTT TGT GCA GGA TTT GGC ATA TAC GAT AGA TGG					
---	---	---	---	---	---
Phe Ser Tyr Ala Tyr Trp Glu Phe Cys Ala Gly Phe Gly Ile Tyr Asp Arg Trp					
1035	1044	1053	1062	1071	1080
TCT CAA AAC TGG ATC GAA CCA TTG GCA ACA GCT GTG GTT GGC ACA GGC AAA GAG					
---	---	---	---	---	---
Ser Gln Asn Trp Ile Glu Pro Leu Ala Thr Ala Val Val Gly Thr Gly Lys Glu					
TAA 3'					
---					
...					

Figure 13b(Continued)



*Thermotoga maritima* Pullulanase (6GP3)

```

      9      18      27      36      45      54
5'  ATG GAT CTT ACA AAG GTG GGG ATC ATA GTG AGG CTG AAC GAG TGG CAG GCA AAA
    -----
    Met Asp Leu Thr Lys Val Gly Ile Ile Val Arg Leu Asn Glu Trp Gln Ala Lys

      63      72      81      90      99     108
    GAC GTG GCA AAA GAC AGG TTC ATA GAG ATA AAA GAC GGA AAG GCT GAA CTG TGG
    -----
    Asp Val Ala Lys Asp Arg Phe Ile Glu Ile Lys Asp Gly Lys Ala Glu Val Trp

     117     126     135     144     153     162
    ATA CTC CAG GGA GTG GAA GAG ATT TTC TAC GAA AAA CCA GAC ACA TCT CCC AGA
    -----
    Ile Leu Gln Gly Val Glu Glu Ile Phe Tyr Glu Lys Pro Asp Thr Ser Pro Arg

     171     180     189     198     207     216
    ATC TTC TTC GCA CAG GCA AGG TCG AAC AAG GTG ATC GAG GCT TTT CTG ACC AAT
    -----
    Ile Phe Phe Ala Gln Ala Arg Ser Asn Lys Val Ile Glu Ala Phe Leu Thr Asn

     225     234     243     252     261     270
    GCT CTG GAT ACG AAA AAG AAA GAA CTC TTC AAG GTT ACT GTT GAC GGA AAA GAG
    -----
    Pro Val Asp Thr Lys Lys Lys Glu Leu Phe Lys Val Thr Val Asp Gly Lys Glu

     279     288     297     306     315     324
    ATT CCC GTC TCA AGA GTG GAA AAG GCC GAT CCC ACG GAC ATA GAC GTG ACG AAC
    -----
    Ile Pro Val Ser Arg Val Glu Lys Ala Asp Pro Thr Asp Ile Asp Val Thr Asn

     333     342     351     360     369     378
    TAC GTG AGA ATC GTC CTT TCT GAA TCC CTG AAA GAA GAA GAC CTC AGA AAA GAC
    -----
    Tyr Val Arg Ile Val Leu Ser Glu Ser Leu Lys Glu Glu Asp Leu Arg Lys Asp

     387     396     405     414     423     432
    GTG GAA CTG ATC ATA GAA GGT TAC AAA CCG GCA AGA GTC ATC ATG ATG GAG ATC
    -----
    Val Glu Leu Ile Ile Glu Gly Tyr Lys Pro Ala Arg Val Ile Met Met Glu Ile

     441     450     459     468     477     486
    CTG GAC GAC TAC TAT TAC GAT GGA GAG CTC GGA GCC GTA TAT TCT CCA GAG AAG
    -----
    Leu Asp Asp Tyr Tyr Tyr Asp Gly Glu Leu Gly Ala Val Tyr Ser Pro Glu Lys

     495     504     513     522     531     540
    ACG ATA TTC AGA GTC TGG TCC CCC GTT TCT AAG TCG GTA AAG GTG CTT CTC TTC
    -----
    Thr Ile Phe Arg Val Trp Ser Pro Val Ser Lys Trp Val Lys Val Leu Leu Phe
  
```

Figure 14a

*Thermotoga maritima* Pullulanase (60P3) (continued)

```

549      558      567      576      585      594
AAA AAC GGA GAA GAC ACA GAA CCG TAC CAG GTT GTG AAC ATG GAA TAC AAG GGA
-----
Lys Asn Gly Glu Asp Thr Glu Pro Tyr Gln Val Val Asn Met Glu Tyr Lys Gly

603      612      621      630      639      648
AAC GGG GTC TGG GAA GCG GTT GTT GAA GGC GAT CTC GAC GGA GTG TTC TAC CTC
-----
Asn Gly Val Trp Glu Ala Val Val Glu Gly Asp Leu Asp Gly Val Phe Tyr Leu

657      666      675      684      693      702
TAT CAG CTC GAA AAC TAC GGA AAG ATC AGA ACA ACC GTC GAT CCT TAT TCG AAA
-----
Tyr Gln Leu Glu Asn Tyr Gly Lys Ile Arg Thr Thr Val Asp Pro Tyr Ser Lys

711      720      729      738      747      756
CCG GTT TAC GCA AAC AAC CAA GAG AGC GCC GTT GTG AAT CTT GCC AGG ACA AAC
-----
Ala Val Tyr Ala Asn Asn Gln Glu Ser Ala Val Val Asn Leu Ala Arg Thr Asn

765      774      783      792      801      810
CCA GAA GGA TGG GAA AAC GAC AGG GGA CCG AAA ATC GAA GGA TAC GAA GAC GCG
-----
Pro Glu Gly Trp Glu Asn Asp Arg Gly Pro Lys Ile Glu Gly Tyr Glu Asp Ala

819      828      837      846      855      864
ATA ATC TAT GAA ATA CAC ATA GCG GAC ATC ACA GGA CTC GAA AAC TCC GGG GTA
-----
Ile Ile Tyr Glu Ile His Ile Ala Asp Ile Thr Gly Leu Glu Asn Ser Gly Val

873      882      891      900      909      918
AAA AAC AAA GGC CTC TAT CTC GGG CTC ACC GAA GAA AAC ACG AAA GGA CCG GCG
-----
Lys Asn Lys Gly Leu Tyr Leu Gly Leu Thr Glu Glu Asn Thr Lys Gly Pro Gly

927      936      945      954      963      972
GGT GTG ACA ACA GGC CTT TCG CAC CTT GTG GAA CTC GGT GTT ACA CAC GTT CAT
-----
Gly Val Thr Thr Gly Leu Ser His Leu Val Glu Leu Gly Val Thr His Val His

981      990      999      1008      1017      1026
ATA CTT CCT TTC TTT GAT TTC TAC ACA GGC GAC GAA CTC GAT AAA GAT TTC GAG
-----
Ile Leu Pro Phe Phe Asp Phe Tyr Thr Gly Asp Glu Leu Asp Lys Asp Phe Glu

1035      1044      1053      1062      1071      1080
AAG TAC TAC AAC TGG GGT TAC GAT CCT TAC CTG TTC ATG GTT CCG GAG GGC ACA
-----
Lys Tyr Tyr Asn Trp Gly Tyr Asp Pro Tyr Leu Phe Met Val Pro Glu Gly Arg

```

Figure 14b(Continued)

*Thermotoga maritima* Pullulanase (6073) (continued)

```

1089      1098      1107      1116      1125      1134
TAC TCA ACC GAT CCC AAA AAC CCA CAC ACG AGA ATC AGA GAA GTC AAA GAA ATG
---
Tyr Ser Thr Asp Pro Lys Asn Pro His Thr Arg Ile Arg Glu Val Lys Glu Met

1143      1152      1161      1170      1179      1188
GTC AAA GCC CTT CAC AAA CAC GGT ATA GGT GTG ATT ATG GAC ATG GTG TTC CCT
---
Val Lys Ala Leu His Lys His Gly Ile Gly Val Ile Met Asp Met Val Phe Pro

1197      1206      1215      1224      1233      1242
CAC ACC TAC GGT ATA GGC GAA CTC TCT GCG TTC GAT CAG ACG GTG CCG TAC TAC
---
His Thr Tyr Gly Ile Gly Glu Leu Ser Ala Phe Asp Gln Thr Val Pro Tyr Tyr

1251      1260      1269      1278      1287      1296
TTC TAC AGA ATC GAC AAG ACA GGT GCC TAT TTG AAC GAA AGC GGA TGT GGT AAC
---
Phe Tyr Arg Ile Asp Lys Thr Gly Ala Tyr Leu Asn Glu Ser Gly Cys Gly Asn

1305      1314      1323      1332      1341      1350
GTC ATC GCA AGC GAA AGA CCC ATG ATG AGA AAA TTC ATA GTC GAT ACC GTC ACC
---
Val Ile Ala Ser Glu Arg Pro Met Met Arg Lys Phe Ile Val Asp Thr Val Thr

1359      1368      1377      1386      1395      1404
TAC TGG GTA AAG GAG TAT CAC ATA GAC GGA TTC AGG TTC GAT CAG ATG GGT CTC
---
Tyr Trp Val Lys Glu Tyr His Ile Asp Gly Phe Arg Phe Asp Gln Met Gly Leu

1413      1422      1431      1440      1449      1458
ATC GAC AAA AAG ACA ATG CTC GAA GTC GAA AGA GCT CTT CAT AAA ATC GAT CCA
---
Ile Asp Lys Lys Thr Met Leu Glu Val Glu Arg Ala Leu His Lys Ile Asp Pro

1467      1476      1485      1494      1503      1512
ACT ATC ATT CTC TAC GGC GAA CCG TGG GGT GGA TGG GCA GCA CCG ATC AGG TTT
---
Thr Ile Ile Leu Tyr Gly Glu Pro Trp Gly Gly Trp Gly Ala Pro Ile Arg Phe

1521      1530      1539      1548      1557      1566
GGA AAG AGC GAT GTC GCC GGC ACA CAC GTG GCA GCT TTC AAC GAT GAG TTC AGA
---
Gly Lys Ser Asp Val Ala Gly Thr His Val Ala Ala Phe Asn Asp Glu Phe Arg

1575      1584      1593      1602      1611      1620
GAC GCA ATA AGG GGT TCC GTG TTC AAC CCG AGC GTC AAG CGA TTC GTC ATG GGA
---
Asp Ala Ile Arg Gly Ser Val Phe Asn Pro Ser Val Lys Gly Phe Val Met Gly

```

Figure 14C(Continued)

*Thermotoga maritima* Pullulanase (6GP3) (continued)

1629	1638	1647	1656	1665	1674
GGA TAC GGA AAG GAA ACC AAG ATC AAA AGG GGT GTT GTT GGA AGC ATA AAC TAC					
---	---	---	---	---	---
Gly Tyr Gly Lys Glu Thr Lys Ile Lys Arg Gly Val Val Gly Ser Ile Asn Tyr					
1683	1692	1701	1710	1719	1728
GAC GGA AAA CTC ATC AAA AGT TTC GCC CTT GAT CCA GAA GAA ACT ATA AAC TAC					
---	---	---	---	---	---
Asp Gly Lys Leu Ile Lys Ser Phe Ala Leu Asp Pro Glu Glu Thr Ile Asn Tyr					
1737	1746	1755	1764	1773	1782
GCA GCG TGT CAC GAC AAC CAC ACA CTG TGG GAC AAG AAC TAC CTT GCC GCC AAA					
---	---	---	---	---	---
Ala Ala Cys His Asp Asn His Thr Leu Trp Asp Lys Asn Tyr Leu Ala Ala Lys					
1791	1800	1809	1818	1827	1836
GCT GAT AAG AAA AAG GAA TGG ACC GAA GAA GAA CTG AAA AAC GCC CAG AAA CTG					
---	---	---	---	---	---
Ala Asp Lys Lys Lys Glu Trp Thr Glu Glu Glu Leu Lys Asn Ala Gln Lys Leu					
1845	1854	1863	1872	1881	1890
GTT GGT GCG ATA CTT CTC ACT TCT CAA GGT GTT CCT TTC CTC CAC GGA GGG CAG					
---	---	---	---	---	---
Ala Gly Ala Ile Leu Leu Thr Ser Gln Gly Val Pro Phe Leu His Gly Gly Gln					
1899	1908	1917	1926	1935	1944
GAC TTC TGC AGG ACG ACG AAT TTC AAC GAC AAC TCC TAC AAC GCC CCT ATC TCG					
---	---	---	---	---	---
Asp Phe Cys Arg Thr Thr Asn Phe Asn Asp Asn Ser Tyr Asn Ala Pro Ile Ser					
1953	1962	1971	1980	1989	1998
ATA AAC GGC TTC GAT TAC GAA AGA AAA CTT CAG TTC ATA GAC GTG TTC AAT TAC					
---	---	---	---	---	---
Ile Asn Gly Phe Asp Tyr Glu Arg Lys Leu Gln Phe Ile Asp Val Phe Asn Tyr					
2007	2016	2025	2034	2043	2052
CAC AAG GGT CTC ATA AAA CTC AGA AAA GAA CAC CCT GCT TTC AGG CTG AAA AAC					
---	---	---	---	---	---
His Lys Gly Leu Ile Lys Leu Arg Lys Glu His Pro Ala Phe Arg Leu Lys Asn					
2061	2070	2079	2088	2097	2106
GCT GAA GAG ATC AAA AAA CAC CTG GAA TTT CTC CCG GGC GGG AGA AGA ATA GTT					
---	---	---	---	---	---
Ala Glu Glu Ile Lys Lys His Leu Glu Phe Leu Pro Gly Gly Arg Arg Ile Val					
2115	2124	2133	2142	2151	2160
GCG TTC ATG CTT AAA GAC CAC GCA GGT GGT GAT CCC TGG AAA GAC ATC GTG GTG					
---	---	---	---	---	---
Ala Phe Met Leu Lys Asp His Ala Gly Gly Asp Pro Trp Lys Asp Ile Val Val					

Figure 14d(Continued)

*Thermotoga maritima* Pullulanase (60P3) (continued)

2169	2178	2187	2196	2205	2214
ATT TAC AAT GCA AAC TTA GAG AAG ACA ACA TAC AAA CTG CCA GAA GGA AAA TGG					
Ile Tyr Asn Gly Asn Leu Glu Lys Thr Thr Tyr Lys Leu Pro Glu Gly Lys Trp					
2223	2232	2241	2250	2259	2268
AAT GTG GTT GTG AAC AGC CAG AAA GCC GGA ACA GAA GTG ATA GAA ACC GTC GAA					
Asn Val Val Val Asn Ser Gln Lys Ala Gly Thr Glu Val Ile Glu Thr Val Glu					
2277	2286	2295	2304	2313	
GGA ACA ATA GAA CTC GAT CCG CTT TCC GCG TAC GTT CTG TAC AGA GAG TGA 3'					
Gly Thr Ile Glu Leu Asp Pro Leu Ser Ala Tyr Val Leu Tyr Arg Glu ...					

Figure 14e(Continued)

Figure 15a *Thermotoga maritima* MSB8 (Clone # 6GP2) Glycosidase

1  
CTT TTA TTG ATC GTT GAG CTC TCT TTC GTT CTC TTT GCA AGT GAC GAG TTC  
Leu Leu Leu Ile Val Glu Leu Ser Phe Val Leu Phe Ala Ser Asp Glu Phe  
GTG AAA GTG GAA AAC GGA AAA TTC GCT CTG AAC GGA AAA GAA TTC AGA TTC  
Val Lys Val Glu Asn Gly Lys Phe Ala Leu Asn Gly Lys Glu Phe Arg Phe  
ATT GGA AGC AAC AAC TAC TAC ATG CAC TAC AAG AGC AAC GGA ATG ATA GAC  
Ile Gly Ser Asn Asn Tyr Tyr Met His Tyr Lys Ser Asn Gly Met Ile Asp  
AGT GTT CTG GAG AGT GCC AGA GAC ATG GGT ATA AAG GTC CTC AGA ATC TGG  
Ser Val Leu Glu Ser Ala Arg Asp Met Gly Ile Lys Val Leu Arg Ile Trp  
GGT TTC CTC GAC GGG GAG AGT TAC TGC AGA GAC AAG AAC ACC TAC ATG CAT  
Gly Phe Leu Asp Gly Glu Ser Tyr Cys Arg Asp Lys Asn Thr Tyr Met His  
CCT GAG CCC GGT GTT TTC GGG GTG CCA GAA GGA ATA TCG AAC GCC CAG AGC  
Pro Glu Pro Gly Val Phe Gly Val Pro Glu Gly Ile Ser Asn Ala Gln Ser  
GGT TTC GAA AGA CTC GAC TAC ACA GTT GCG AAA GCG AAA GAA CTC GGT ATA  
Gly Phe Glu Arg Leu Asp Tyr Thr Val Ala Lys Ala Lys Glu Leu Gly Ile  
AAA CTT GTC ATT GTT CTT GTG AAC AAC TGG GAC GAC TTC GGT GGA ATG AAC  
Lys Leu Val Ile Val Leu Val Asn Asn Trp Asp Asp Phe Gly Gly Met Asn  
CAG TAC GTG AGG TGG TTT GGA GGA ACC CAT CAC GAC GAT TTC TAC AGA GAT  
Gln Tyr Val Arg Trp Phe Gly Gly Thr His His Asp Asp Phe Tyr Arg Asp  
GAG AAG ATC AAA GAA GAG TAC AAA AAG TAC GTC TCC TTT CTC GTA AAC CAT  
Glu Lys Ile Lys Glu Glu Tyr Lys Lys Tyr Val Ser Phe Leu Val Asn His  
GTC AAT ACC TAC ACG GGA GTT CCT TAC AGG GAA GAG CCC ACC ATC ATG GCC  
Val Asn Thr Tyr Thr Gly Val Pro Tyr Arg Glu Glu Pro Thr Ile Met Ala  
TGG GAG CTT GCA AAC GAA CCG CGC TGT GAG ACG GAC AAA TCG GGG AAC ACG  
Trp Glu Leu Ala Asn Glu Pro Arg Cys Glu Thr Asp Lys Ser Gly Asn Thr

CTC GTT GAG TGG GTG AAG GAG ATG AGC TCC TAC ATA AAG AGT CTG GAT CCC  
Leu Val Glu Trp Val Lys Glu Met Ser Ser Tyr Ile Lys Ser Leu Asp Pro

AAC CAC CTC GTG GCT GTG GGG GAC GAA GGA TTC TTC AGC AAC TAC GAA GGA  
Asn His Leu Val Ala Val Gly Asp Glu Gly Phe Phe Ser Asn Tyr Glu Gly

TTC AAA CCT TAC GGT GGA GAA GCC GAG TGG GCC TAC AAC GGC TGG TCC GGT  
Phe Lys Pro Tyr Gly Gly Glu Ala Glu Trp Ala Tyr Asn Gly Trp Ser Gly

GTT GAC TGG AAG AAG CTC CTT TCG ATA GAG ACG GTG GAC TTC GGC ACG TTC  
Val Asp Trp Lys Lys Leu Leu Ser Ile Glu Thr Val Asp Phe Gly Thr Phe

CAC CTC TAT CCG TCC CAC TGG GGT GTC AGT CCA GAG AAC TAT GCC CAG TGG  
His Leu Tyr Pro Ser His Trp Gly Val Ser Pro Glu Asn Tyr Ala Gln Trp

GGA GCG AAG TGG ATA GAA GAC CAC ATA AAG ATC GCA AAA GAG ATC GGA AAA  
Gly Ala Lys Trp Ile Glu Asp His Ile Lys Ile Ala Lys Glu Ile Gly Lys

CCC GTT GTT CTG GAA GAA TAT GGA ATT CCA AAG AGT GCG CCA GTT AAC AGA  
Pro Val Val Leu Glu Glu Tyr Gly Ile Pro Lys Ser Ala Pro Val Asn Arg

ACG GCC ATC TAC AGA CTC TGG AAC GAT CTG GTC TAC GAT CTC GGT GGA GAT  
Thr Ala Ile Tyr Arg Leu Trp Asn Asp Leu Val Tyr Asp Leu Gly Gly Asp

GGA GCG ATG TTC TGG ATG CTC GCG GGA ATC GGG GAA GGT TCG GAC AGA GAC  
Gly Ala Met Phe Trp Met Leu Ala Gly Ile Gly Glu Gly Ser Asp Arg Asp

GAG AGA GGG TAC TAT CCG GAC TAC GAC GGT TTC AGA ATA GTG AAC GAC GAC  
Glu Arg Gly Tyr Tyr Pro Asp Tyr Asp Gly Phe Arg Ile Val Asn Asp Asp

AGT CCA GAA GCG GAA CTG ATA AGA GAA TAC GCG AAG CTG TTC AAC ACA GGT  
Ser Pro Glu Ala Glu Leu Ile Arg Glu Tyr Ala Lys Leu Phe Asn Thr Gly

GAA GAC ATA AGA GAA GAC ACC TGC TCT TTC ATC CTT CCA AAA GAC GGC ATG  
Glu Asp Ile Arg Glu Asp Thr Cys Ser Phe Ile Leu Pro Lys Asp Gly Met

GAG ATC AAA AAG ACC GTG GAA GTG AGG GCT GGT GTT TTC GAC TAC AGC AAC

Figure 15b (continued)

Glu Ile Lys Lys Thr Val Glu Val Arg Ala Gly Val Phe Asp Tyr Ser Asn  
ACG TTT GAA AAG TTG TCT GTC AAA GTC GAA GAT CTG GTT TTT GAA AAT GAG  
Thr Phe Glu Lys Leu Ser Val Lys Val Glu Asp Leu Val Phe Glu Asn Glu  
ATA GAG CAT CTC GGA TAC GGA ATT TAC GGC TTT GAT CTC GAC ACA ACC CGG  
Ile Glu His Leu Gly Tyr Gly Ile Tyr Gly Phe Asp Leu Asp Thr Thr Arg  
ATC CCG GAT GGA GAA CAT GAA ATG TTC CTT GAA GGC CAC TTT CAG GGA AAA  
Ile Pro Asp Gly Glu His Glu Met Phe Leu Glu Gly His Phe Gln Gly Lys  
ACG GTG AAA GAC TCT ATC AAA GCG AAA GTG GTG AAC GAA GCA CGG TAC GTG  
Thr Val Lys Asp Ser Ile Lys Ala Lys Val Val Asn Glu Ala Arg Tyr Val  
CTC GCA GAG GAA GTT GAT TTT TCC TCT CCA GAA GAG GTG AAA AAC TGG TGG  
Leu Ala Glu Glu Val Asp Phe Ser Ser Pro Glu Glu Val Lys Asn Trp Trp  
AAC AGC GGA ACC TGG CAG GCA GAG TTC GGG TCA CCT GAC ATT GAA TGG AAC  
Asn Ser Gly Thr Trp Gln Ala Glu Phe Gly Ser Pro Asp Ile Glu Trp Asn  
GGT GAG GTG GGA AAT GGA GCA CTG CAG CTG AAC GTG AAA CTG CCC GGA AAG  
Gly Glu Val Gly Asn Gly Ala Leu Gln Leu Asn Val Lys Leu Pro Gly Lys  
AGC GAC TGG GAA GAA GTG AGA GTA GCA AGG AAG TTC GAA AGA CTC TCA GAA  
Ser Asp Trp Glu Glu Val Arg Val Ala Arg Lys Phe Glu Arg Leu Ser Glu  
TGT GAG ATC CTC GAG TAC GAC ATC TAC ATT CCA AAC GTC GAG GGA CTC AAG  
Cys Glu Ile Leu Glu Tyr Asp Ile Tyr Ile Pro Asn Val Glu Gly Leu Lys  
GGA AGG TTG AGG CCG TAC GCG GTT CTG AAC CCC GGC TGG GTG AAG ATA GGC  
Gly Arg Leu Arg Pro Tyr Ala Val Leu Asn Pro Gly Trp Val Lys Ile Gly  
CTC GAC ATG AAC AAC GCG AAC GTG GAA AGT GCG GAG ATC ATC ACT TTC GGC  
Leu Asp Met Asn Asn Ala Asn Val Glu Ser Ala Glu Ile Ile Thr Phe Gly  
GGA AAA GAG TAC AGA AGA TTC CAT GTA AGA ATT GAG TTC GAC AGA ACA GCG  
Gly Lys Glu Tyr Arg Arg Phe His Val Arg Ile Glu Phe Asp Arg Thr Ala

Figure 15C(continued)



GGG GTG AAA GAA CTT CAC ATA GGA GTT GTC GGT GAT CAT CTG AGG TAC GAT  
Gly Val Lys Glu Leu His Ile Gly Val Val Gly Asp His Leu Arg Tyr Asp

GGA CCG ATT TTC ATC GAT AAT GTG AGA CTT TAT AAA AGA ACA GGA GGT ATG  
Gly Pro Ile Phe Ile Asp Asn Val Arg Leu Tyr Lys Arg Thr Gly Gly Met

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END

Figure 15d(continued)

Figure No. 16 *Thermotoga maritima* MSB8(6gb4)

1	ATG AAA AGA ATC GAC CTG AAT GGT TTC TGG AGC GTT AGG GAT AAC GAA GGG AGA TTT TCG	60
1	Met Lys Arg Ile Asp Leu Asn Gly Phe Trp Ser Val Arg Asp Asn Glu Gly Arg Phe Ser	20
61	TTT GAA GGG ACT GTG CCA GGG GTT GTC CAG GCA GAT CTG GTC AGA AAA GGT CTT CTT CCA	120
21	Phe Glu Gly Thr Val Pro Gly Val Val Gln Ala Asp Leu Val Arg Lys Gly Leu Leu Pro	40
121	CAC CCG TAC GTT GGG ATG AAC GAA GAT CTC TTC AAG GAA ATA GAA GAC AGA GAG TGG ATC	180
41	His Pro Tyr Val Gly Met Asn Glu Asp Leu Phe Lys Glu Ile Glu Asp Arg Glu Trp Ile	60
181	TAC GAG AGG GAG TTC GAG TTC AAA GAA GAT GTG AAA GAG GGG GAA CGT GTC GAT CTC GTT	240
61	Tyr Glu Arg Glu Phe Glu Phe Lys Glu Asp Val Lys Glu Gly Glu Arg Val Asp Leu Val	80
241	TTT GAG GGC GTC GAC ACG CTG TCG GAT GTT TAT CTG AAC GGT GTT TAC CTT GGA AGC ACC	300
81	Phe Glu Gly Val Asp Thr Leu Ser Asp Val Tyr Leu Asn Gly Val Tyr Leu Gly Ser Thr	100
301	GAA GAC ATG TTC ATC GAG TAT CGC TTC GAT GTC ACG AAC GTG TTG AAA GAA AAG AAT CAC	360
101	Glu Asp Met Phe Ile Glu Tyr Arg Phe Asp Val Thr Asn Val Leu Lys Glu Lys Asn His	120
361	CTG AAG GTG TAC ATA AAA TCT CCC ATC AGA GTT CCG AAA ACT CTC GAG CAG AAC TAC GGG	420
121	Leu Lys Val Tyr Ile Lys Ser Pro Ile Arg Val Pro Lys Thr Leu Glu Gln Asn Tyr Gly	140
421	GTC CTC GGC GGT CCT GAA GAT CCC ATC AGA GGA TAC ATA AGA AAA GCC CAG TAT TCG TAC	480
141	Val Leu Gly Gly Pro Glu Asp Pro Ile Arg Gly Tyr Ile Arg Lys Ala Gln Tyr Ser Tyr	160
481	GGA TGG GAC TGG GGT GCC AGA ATC GTT ACA AGC GGT ATT TGG AAA CCC GTC TAC CTC GAG	540
161	Gly Trp Asp Trp Gly Ala Arg Ile Val Thr Ser Gly Ile Trp Lys Pro Val Tyr Leu Glu	180
541	GTG TAC AGG GCA CGT CTT CAG GAT TCA ACG GCT TAT CTG TTG GAA CTT GAG GGG AAA GAT	600
181	Val Tyr Arg Ala Arg Leu Gln Asp Ser Thr Ala Tyr Leu Leu Glu Leu Glu Gly Lys Asp	200
601	GCC CTT GTG AGG GTG AAC GGT TTC GTA CAC GGG GAA GGA AAT CTC ATT GTG GAA GTT TAT	660
201	Ala Leu Val Arg Val Asn Gly Phe Val His Gly Glu Gly Asn Leu Ile Val Glu Val Tyr	220
661	GTA AAC GGT GAA AAG ATA GGG GAG TTT CCT GTT CTT GAA AAG AAC GGA GAA AAG CTC TTC	720
221	Val Asn Gly Glu Lys Ile Gly Glu Phe Pro Val Leu Glu Lys Asn Gly Glu Lys Leu Phe	240
721	GAT GGA GTG TTC CAC CTG AAA GAT GTG AAA CTA TGG TAT CCG TGG AAC GTG GGG AAA CCG	780
241	Asp Gly Val Phe His Leu Lys Asp Val Lys Leu Trp Tyr Pro Trp Asn Val Gly Lys Pro	260

781	TAC CTG TAC GAT TTC GTT TTC GTG TTG AAA GAC TTA AAC GGA GAG ATC TAC AGA GAA GAA	840
261	Tyr Leu Tyr Asp Phe Val Phe Val Leu Lys Asp Leu Asn Gly Glu Ile Tyr Arg Glu Glu	280
841	AAG AAA ATC GGT TTG AGA AGA GTC AGA ATC GTT CAG GAG CCC GAT GAA GAA GGA AAA ACT	900
281	Lys Lys Ile Gly Leu Arg Arg Val Arg Ile Val Gln Glu Pro Asp Glu Glu Gly Lys Thr	300
901	TTC ATA TTC GAA ATC AAC GGT GAG AAA GTC TTC GCT AAG GGT GCT AAC TGG ATT CCC TCA	960
301	Phe Ile Phe Glu Ile Asn Gly Glu Lys Val Phe Ala Lys Gly Ala Asn Trp Ile Pro Ser	320
961	GAA AAC ATC CTC ACG TGG TTG AAG GAG GAA GAT TAC GAA AAG CTC GTC AAA ATG GCA AGG	1020
321	Glu Asn Ile Leu Thr Trp Leu Lys Glu Glu Asp Tyr Glu Lys Leu Val Lys Met Ala Arg	340
1021	AGT GCC AAT ATG AAC ATG CTC AGG GTC TGG GGA GGA GGA ATC TAC GAG AGA GAG ATC TTC	1080
341	Ser Ala Asn Met Asn Met Leu Arg Val Trp Gly Gly Gly Ile Tyr Glu Arg Glu Ile Phe	360
1081	TAC AGA CTC TGT GAT GAA CTC GGT ATC ATG GTG TGG CAG GAT TTC ATG TAC GCG TGT CTT	1140
361	Tyr Arg Leu Cys Asp Glu Leu Gly Ile Met Val Trp Gln Asp Phe Met Tyr Ala Cys Leu	380
1141	GAA TAT CCG GAT CAT CTT CCG TGG TTC AGA AAA CTC GCG AAC GAA GAG GCA AGA AAG ATT	1200
381	Glu Tyr Pro Asp His Leu Pro Trp Phe Arg Lys Leu Ala Asn Glu Glu Ala Arg Lys Ile	400
1201	GTG AGA AAA CTC AGA TAC CAT CCC TCC ATT GTT CTC TGG TGC GGA AAC AAC GAA AAC AAC	1260
401	Val Arg Lys Leu Arg Tyr His Pro Ser Ile Val Leu Trp Cys Gly Asn Asn Glu Asn Asn	420
1261	TGG GGA TTC GAT GAA TGG GGA AAT ATG GCC AGA AAA GTG GAT GGT ATC AAC CTC GGA AAC	1320
421	Trp Gly Phe Asp Glu Trp Gly Asn Met Ala Arg Lys Val Asp Gly Ile Asn Leu Gly Asn	440
1321	AGG CTC TAC CTC TTC GAT TTT CCT GAG ATT TGT GCC GAA GAA GAC CCG TCC ACT CCC TAT	1380
441	Arg Leu Tyr Leu Phe Asp Phe Pro Glu Ile Cys Ala Glu Glu Asp Pro Ser Thr Pro Tyr	460
1381	TGG CCA TCC AGT CCA TAC GGC GGT GAA AAA GCG AAC AGC GAA AAG GAA GGA GAC AGG CAC	1440
461	Trp Pro Ser Ser Pro Tyr Gly Gly Glu Lys Ala Asn Ser Glu Lys Glu Gly Asp Arg His	480
1441	GTC TGG TAC GTG TGG AGT GGC TGG ATG AAC TAC GAA AAC TAC GAA AAA GAC ACC GGA AGG	1500
481	Val Trp Tyr Val Trp Ser Gly Trp Met Asn Tyr Glu Asn Tyr Glu Lys Asp Thr Gly Arg	500
1501	TTC ATC AGC GAG TTT GGA TTT CAG GGT GCT CCC CAT CCA GAG ACG ATA GAG TTC TTT TCA	1560
501	Phe Ile Ser Glu Phe Gly Phe Gln Gly Ala Pro His Pro Glu Thr Ile Glu Phe Phe Ser	520
1561	AAA CCC GAG GAA AGA GAG ATA TTC CAT CCC GTC ATG CTG AAG CAC AAC AAA CAG GTG GAA	1620
521	Lys Pro Glu Glu Arg Glu Ile Phe His Pro Val Met Leu Lys His Asn Lys Gln Val Glu	540

Figure 16b(continued)

1621	GGA CAG GAA AGA TTG ATC AGG TTC ATA TTC GGA AAT TTT GGA AAG TGT AAA GAT TTC GAC	1680
541	Gly Gln Glu Arg Leu Ile Arg Phe Ile Phe Gly Asn Phe Gly Lys Cys Lys Asp Phe Asp	560
1681	AGT TTT GTG TAT CTG TCC CAG CTC AAC CAG GCG GAG GCG ATC AAG TTC GGT GTT GAA CAC	1740
561	Ser Phe Val Tyr Leu Ser Gln Leu Asn Gln Ala Glu Ala Ile Lys Phe Gly Val Glu His	580
1741	TGG CGA AGC AGG AAG TAC AAA ACG GCC GGC GCT CTC TTC TGG CAG TTC AAC GAC AGC TGG	1800
581	Trp Arg Ser Arg Lys Tyr Lys Thr Ala Gly Ala Leu Phe Trp Gln Phe Asn Asp Ser Trp	600
1801	CCG GTC TTC AGC TGG TCC GCA GTC GAT TAC TTC AAA AGG CCC AAA GCT CTC TAC TAC TAT	1860
601	Pro Val Phe Ser Trp Ser Ala Val Asp Tyr Phe Lys Arg Pro Lys Ala Leu Tyr Tyr Tyr	620
1861	GCG AGA AGA TTC TTC GCT GAA GTT CTA CCC GTT TTG AAG AAG AGA GAC AAC AAA ATA GAA	1920
621	Ala Arg Arg Phe Phe Ala Glu Val Leu Pro Val Leu Lys Lys Arg Asp Asn Lys Ile Glu	640
1921	CTG CTG GTG GGT GAG CGA TCT GAG GGA GAC AAA AGA AGT CTC TCT CAG GCT TGC AGC CTA	1980
641	Leu Leu Val Gly Glu Arg Ser Glu Gly Asp Lys Arg Ser Leu Ser Gln Ala Cys Ser Leu	660
1981	CGA GAA GAA GGG AGA AAA GGT ATT CGA AAA GAC TTA CAG AAC GGT ACT CCC AGC AGA CGG	2040
661	Arg Glu Glu Gly Arg Lys Gly Ile Arg Lys Asp Leu Gln Asn Gly Thr Pro Ser Arg Arg	680
2041	TGT GAG TTT GGT TGA	2055
681	Cys Glu Phe Gly End	685

Figure 16C(continued)

Figure No. 12-Bankia gouldi (37gp4)

1	ATG AAA AAA AAT CTA CTA ATG TTT AAA AGG CTT ACG TAT CTA CCT TTG TTT TTA ATG CTG	60
1	Met Lys Lys Asn Leu Leu Met Phe Lys Arg Leu Thr Tyr Leu Pro Leu Phe Leu Met Leu	20
61	CTC TCA CTA AGT TCA GTA GCT CAA TCT CCT GTA GAA AAA CAT GGC CGT TTA CAA GTT GAC	120
21	Leu Ser Leu Ser Ser Val Ala Gln Ser Pro Val Glu Lys His Gly Arg Leu Gln Val Asp	40
121	GGA AAC CGC ATT CTT AAT GCG TCT GGA GAA ATT ACG AGC TTA GCT GGT AAC AGC CTC TTT	180
41	Gly Asn Arg Ile Leu Asn Ala Ser Gly Glu Ile Thr Ser Leu Ala Gly Asn Ser Leu Phe	60
181	TGG AGT AAT GCT GGA GAC ACC TCC GAT TTT TAT AAT GCA GAA ACT GTT GAT TTT TTA GCA	240
61	Trp Ser Asn Ala Gly Asp Thr Ser Asp Phe Tyr Asn Ala Glu Thr Val Asp Phe Leu Ala	80
241	GAA AAC TGG AAT AGC TCA CTT ATT AGA ATA GCT ATG GGC GTA AAA GAA AAT TGG GAT GGC	300
81	Glu Asn Trp Asn Ser Ser Leu Ile Arg Ile Ala Met Gly Val Lys Glu Asn Trp Asp Gly	100
301	GGA AAT GGC TAT ATT GAT AGT CCG CAG GAG CAA GAA GCT AAA ATT AGA AAA GTT ATT GAT	360
101	Gly Asn Gly Tyr Ile Asp Ser Pro Gln Glu Gln Glu Ala Lys Ile Arg Lys Val Ile Asp	120
361	GCA GCT ATT GCT AAC GGC ATA TAT GTA ATA ATA GAC TGG CAC ACT CAC GAA GCA GAG TTA	420
121	Ala Ala Ile Ala Asn Gly Ile Tyr Val Ile Ile Asp Trp His Thr His Glu Ala Glu Leu	140
421	TAC ACA GAT GAG GCT GTT GAC TTT TTT ACC AGA ATG GCA GAC CTA TAC GGA GAT ACT CCC	480
141	Tyr Thr Asp Glu Ala Val Asp Phe Phe Thr Arg Met Ala Asp Leu Tyr Gly Asp Thr Pro	160
481	AAT GTA ATG TAT GAA ATT TAT AAC GAG CCT ATA TAC CAA AGT TGG CCT GTT ATT AAG AAT	540
161	Asn Val Met Tyr Glu Ile Tyr Asn Glu Pro Ile Tyr Gln Ser Trp Pro Val Ile Lys Asn	180
541	TAT GCA GAG CAA GTA ATT GCT GGT ATA CGT TCT AAA GAC CCA GAT AAT TTA ATA ATT GTA	600
181	Tyr Ala Glu Gln Val Ile Ala Gly Ile Arg Ser Lys Asp Pro Asp Asn Leu Ile Ile Val	200
601	GGT ACT AGC AAT TAT TCT CAG CAA GTT GAT GTA GCA TCA GCA GAC CCA ATA TCT GAT ACT	660
201	Gly Thr Ser Asn Tyr Ser Gln Gln Val Asp Val Ala Ser Ala Asp Pro Ile Ser Asp Thr	220
661	AAT GTG GCA TAT ACT TTA CAT TTT TAT GCA GCA TTT AAC CCG CAT GAT AAC TTA AGA AAT	720
221	Asn Val Ala Tyr Thr Leu His Phe Tyr Ala Ala Phe Asn Pro His Asp Asn Leu Arg Asn	240
721	GTA GCA CAG ACA GCA TTA GAT AAT AAT GTT GCT TTG TTT GTT ACA GAA TGG GGT ACA ATT	780
241	Val Ala Gln Thr Ala Leu Asp Asn Asn Val Ala Leu Phe Val Thr Glu Trp Gly Thr Ile	260

781	TTA AAT ACC GGA CAA GGA GAA CCA GAC AAA GAA AGC ACT AAT ACT TGG ATG GCC TTT TTG	840
261	Leu Asn Thr Gly Gln Gly Glu Pro Asp Lys Glu Ser Thr Asn Thr Trp Met Ala Phe Leu	280
841	AAA GAA AAA GGT ATA AGT CAC GCT AAT TGG TCT TTG AGT GAC AAA GCT TTT CCT GAA ACA	900
281	Lys Glu Lys Gly Ile Ser His Ala Asn Trp Ser Leu Ser Asp Lys Ala Phe Pro Glu Thr	300
901	GGG TCT GTA GTT CAA GCA GGA CAA GGT GTA TCT GGT TTA ATT AGC AAT AAA CTT ACA GCC	960
301	Gly Ser Val Val Gln Ala Gly Gln Gly Val Ser Gly Leu Ile Ser Asn Lys Leu Thr Ala	320
961	TCT GGT GAA ATT GTA AAA AAC ATC ATC CAA AAC TGG GAT ACA GAG ACC TCT ACA GGA CCT	1020
321	Ser Gly Glu Ile Val Lys Asn Ile Ile Gln Asn Trp Asp Thr Glu Thr Ser Thr Gly Pro	340
1021	AAA ACA ACA CAA TGT AGT ACT ATA GAA TGT ATT AGA GCT GCA ATG GAA ACA GCA CAA GCA	1080
341	Lys Thr Thr Gln Cys Ser Thr Ile Glu Cys Ile Arg Ala Ala Met Glu Thr Ala Gln Ala	360
1081	GGA GAT GAA ATT ATA ATT GCC CCT GGA AAC TAC AAT TTT CAA GAC AAG ATA CAA GGT GCC	1140
361	Gly Asp Glu Ile Ile Ile Ala Pro Gly Asn Tyr Asn Phe Gln Asp Lys Ile Gln Gly Ala	380
1141	TTT AAC CGT AGT GTT TAC CTT TAT GGT AGT GCT AAC GGA AAC AGT ACA AAC CCT ATT ATA	1200
381	Phe Asn Arg Ser Val Tyr Leu Tyr Gly Ser Ala Asn Gly Asn Ser Thr Asn Pro Ile Ile	400
1201	TTA AGA GGC GAA AGC GCT ACA AAC CCT CCT GTT TTC TCA GGA TTA GAT TAT AAC AAT GGC	1260
401	Leu Arg Gly Glu Ser Ala Thr Asn Pro Pro Val Phe Ser Gly Leu Asp Tyr Asn Asn Gly	420
1261	TAC CTA TTA AGT ATT GAA GGT GAT TAT TGG AAT ATT AAA GAT ATA GAG TTT AAA ACT GGG	1320
421	Tyr Leu Leu Ser Ile Glu Gly Asp Tyr Trp Asn Ile Lys Asp Ile Glu Phe Lys Thr Gly	440
1321	TCT AAA GGT ATT GTT CTT GAC AAT TCT AAT GGT AGT AAA TTA AAA AAC CTT GTT GTT CAT	1380
441	Ser Lys Gly Ile Val Leu Asp Asn Ser Asn Gly Ser Lys Leu Lys Asn Leu Val Val His	460
1381	GAT ATT GGA GAA GAA GCT ATT CAC TTG CGT GAT GGA TCT AGC AAT AAT AGT ATA GAT GGT	1440
461	Asp Ile Gly Glu Glu Ala Ile His Leu Arg Asp Gly Ser Ser Asn Asn Ser Ile Asp Gly	480
1441	TGC ACT ATA TAC AAT ACA GGT AGA ACT AAA CCT GGT TTT GGT GAA GGT TTA TAT GTA GGC	1500
481	Cys Thr Ile Tyr Asn Thr Gly Arg Thr Lys Pro Gly Phe Gly Glu Gly Leu Tyr Val Gly	500
1501	TCA GAT AAA GGA CAA CAT GAC ACT TAT GAA AGA GCT TGT AAC AAT AAC ACT ATT GAA AAC	1560
501	Ser Asp Lys Gly Gln His Asp Thr Tyr Glu Arg Ala Cys Asn Asn Asn Thr Ile Glu Asn	520
1561	TGT ACC GTT GGA CCC AAT GTA ACA GCA GAA GGC GTA GAT GTT AAG GAA GGT ACA ATG AAC	1620
521	Cys Thr Val Gly Pro Asn Val Thr Ala Glu Gly Val Asp Val Lys Glu Gly Thr Met Asn	540

Figure 17b(continued)

1621	ACT ATT ATA AGA AAT TGC GTG TTT TCT GCA GAA GGA ATT TCA GGA GAA AAT AGC TCA GAT	1680
541	Thr Ile Ile Arg Asn Cys Val Phe Ser Ala Glu Gly Ile Ser Gly Glu Asn Ser Ser Asp	560
1681	GCT TTT ATT GAT TTA AAA GGA GCC TAT GGT TTT GTA TAC AGA AAC ACG TTT AAT GTT GAT	1740
561	Ala Phe Ile Asp Leu Lys Gly Ala Tyr Gly Phe Val Tyr Arg Asn Thr Phe Asn Val Asp	580
1741	GGT TCT GAA GTA ATA AAT ACT GGA GTA GAC TTT TTA GAT AGA GGT ACA GGA TTT AAT ACA	1800
581	Gly Ser Glu Val Ile Asn Thr Gly Val Asp Phe Leu Asp Arg Gly Thr Gly Phe Asn Thr	600
1801	GGT TTT AGA AAT GCA ATA TTT GAA AAT ACA TAT AAC CTT GGC AGT AGA GCT TCA GAA ATT	1860
601	Gly Phe Arg Asn Ala Ile Phe Glu Asn Thr Tyr Asn Leu Gly Ser Arg Ala Ser Glu Ile	620
1861	TCA ACT GCT CGT AAA AAA CAA GGT TCT CCT GAA CAA ACT CAC GTT TGG GAT AAT ATT AGA	1920
621	Ser Thr Ala Arg Lys Lys Gln Gly Ser Pro Glu Gln Thr His Val Trp Asp Asn Ile Arg	640
1921	AAC CCT AAT TCT GTT GAT TTT CCA ATA AGT GAT GGT ACA GAA AAT CTA GTA AAT AAA TTC	1980
641	Asn Pro Asn Ser Val Asp Phe Pro Ile Ser Asp Gly Thr Glu Asn Leu Val Asn Lys Phe	660
1981	TGC CCA GAT TGG AAT ATA GAA CCA TGT AAT CCT GTA GAC GAA ACC AAC CAA GCA CCT ACA	2040
661	Cys Pro Asp Trp Asn Ile Glu Pro Cys Asn Pro Val Asp Glu Thr Asn Gln Ala Pro Thr	680
2041	ATA AGC TTC CTA TCT CCT GTT AAC AAT ATT ACT TTA GTT GAA GGT TAT AAT TTA CAA GTT	2100
681	Ile Ser Phe Leu Ser Pro Val Asn Asn Ile Thr Leu Val Glu Gly Tyr Asn Leu Gln Val	700
2101	GAA GTT AAT GCT ACT GAT GCA GAT GGA ACT ATT GAT AAT GTA AAA CTT TAT ATA GAT AAC	2160
701	Glu Val Asn Ala Thr Asp Ala Asp Gly Thr Ile Asp Asn Val Lys Leu Tyr Ile Asp Asn	720
2161	AAT TTA GTT AGG CAA ATA AAT TCT ACT TCA TAT AAA TGG GGC CAT TCT GAT TCT CCA AAT	2220
721	Asn Leu Val Arg Gln Ile Asn Ser Thr Ser Tyr Lys Trp Gly His Ser Asp Ser Pro Asn	740
2221	ACA GAT GAA CTT AAT GGT CTT ACA GAA GGA ACT TAT ACC TTA AAA GCA ATT GCA ACT GAT	2280
741	Thr Asp Glu Leu Asn Gly Leu Thr Glu Gly Thr Tyr Thr Leu Lys Ala Ile Ala Thr Asp	760
2281	AAC GAC GGG GCT TCT ACA GAA ACG CAA TTT ACG TTA ACT GTA ATA ACA GAA CAA AGT CCG	2340
761	Asn Asp Gly Ala Ser Thr Glu Thr Gln Phe Thr Leu Thr Val Ile Thr Glu Gln Ser Pro	780
2341	TCT GAG AAT TGT GAC TTT AAT ACA CCT TCT TCA ACT GGT TTA GAA GAT TTT GAC ATT AAA	2400
781	Ser Glu Asn Cys Asp Phe Asn Thr Pro Ser Ser Thr Gly Leu Glu Asp Phe Asp Ile Lys	800
2401	AAG TTT TCT AAC GTT TTT GAG TTA GGA TCT GGC GGA CCA TCT TTA AGT AAT TTA AAA ACA	2460

Figure 17c(continued)

801 Lys Phe Ser Asn Val Phe Glu Leu Gly Ser Gly Gly Pro Ser Leu Ser Asn Leu Lys Thr 820  
 2461 TTT ACT ATT AAT TGG AAT TCG CAA TAC AAT GGG TTA TAT CAA TTT TCA ATA AAC ACA AAC 2520  
 821 Phe Thr Ile Asn Trp Asn Ser Gln Tyr Asn Gly Leu Tyr Gln Phe Ser Ile Asn Thr Asn 840  
 2521 AAC GGT GTA CCT GAT TAT TAT ATA AAT TTA AAA CCA AAA ATT ACC TTT CAG TTT AAA AAT 2580  
 841 Asn Gly Val Pro Asp Tyr Tyr Ile Asn Leu Lys Pro Lys Ile Thr Phe Gln Phe Lys Asn 860  
 2581 GCA AAT CCA GAA ATA TCT ATT AGC AAT AGC TTA ATT CCT AAT TTT GAT GGT GAT TAC TGG 2640  
 861 Ala Asn Pro Glu Ile Ser Ile Ser Asn Ser Leu Ile Pro Asn Phe Asp Gly Asp Tyr Trp 880  
 2641 GTA ACA TCA GAT AAC GGT AAT TTT GTG ATG GTA TCT AAA ACT AAT AAT TTT ACG ATA TAC 2700  
 881 Val Thr Ser Asp Asn Gly Asn Phe Val Met Val Ser Lys Thr Asn Asn Phe Thr Ile Tyr 900  
 2701 TTT AGT AAT GAC GCT ACT GCT CCT ATT TGT AAT GTT ACG CCT AGT AAC CAA ATA AGT AAA 2760  
 901 Phe Ser Asn Asp Ala Thr Ala Pro Ile Cys Asn Val Thr Pro Ser Asn Gln Ile Ser Lys 920  
 2761 ATT ACT GAT GAT TCT AGT ATT AAT TTT AAG CTT TAC CCT AAT CCT GCT TTA GAC GAA ACT 2820  
 921 Ile Thr Asp Asp Ser Ser Ile Asn Phe Lys Leu Tyr Pro Asn Pro Ala Leu Asp Glu Thr 940  
 2821 ATT TTT GTG AGC GCT GAA GAT GAA AAA CTA GCT TTG GTG CTT GTA CCA GT 2870  
 941 Ile Phe Val Ser Ala Glu Asp Glu Lys Leu Ala Leu Val Leu Val Pro 956

Figure 17d(continued)



Figure No. 18a *Pyrococcus furiosus* VC1(7EG1)

leader sequence: amino acids 1-24

5' ATG AGC AAG AAA AAG TTC GTC ATC GTA TCT ATC TTA ACA ATC CTT TTA GTA CAG  
 Met Ser Lys Lys Lys Phe Val Ile Val Ser Ile Leu Thr Ile Leu Leu Val Gln

63 72 81 90 99 108  
 GCA ATA TAT TTT GTA GAA AAG TAT CAT ACC TCT GAG GAC AAG TCA ACT TCA AAT  
 Ala Ile Tyr Phe Val Glu Lys Tyr His Thr Ser Glu Asp Lys Ser Thr Ser Asn

117 126 135 144 153 162  
 ACC TCA TCT ACA CCA CCC CAA ACA ACA CTT TCC ACT ACC AAG GTT CTC AAG ATT  
 Thr Ser Ser Thr Pro Pro Gln Thr Thr Leu Ser Thr Thr Lys Val Leu Lys Ile

171 180 189 198 207 216  
 AGA TAC CCT GAT GAC GGT GAG TGG CCA GGA GCT CCT ATT GAT AAG GAT GGT GAT  
 Arg Tyr Pro Asp Asp Gly Glu Trp Pro Gly Ala Pro Ile Asp Lys Asp Gly Asp

225 234 243 252 261 270  
 GGG AAC CCA GAA TTC TAC ATT GAA ATA AAC CTA TGG AAC ATT CTT AAT GCT ACT  
 Gly Asn Pro Glu Phe Tyr Ile Glu Ile Asn Leu Trp Asn Ile Leu Asn Ala Thr

279 288 297 306 315 324  
 GGA TTT GCT GAG ATG ACG TAC AAT TTA ACC AGC GGC GTC CTT CAC TAC GTC CAA  
 Gly Phe Ala Glu Met Thr Tyr Asn Leu Thr Ser Gly Val Leu His Tyr Val Gln

333 342 351 360 369 378  
 CAA CTT GAC AAC ATT GTC TTG AGG GAT AGA AGT AAT TGG GTG CAT GGA TAC CCC  
 Gln Leu Asp Asn Ile Val Leu Arg Asp Arg Ser Asn Trp Val His Gly Tyr Pro

387 396 405 414 423 432  
 GAA ATA TTC TAT GGA AAC AAG CCA TGG AAT GCA AAC TAC GCA ACT GAT GGC CCA  
 Glu Ile Phe Tyr Gly Asn Lys Pro Trp Asn Ala Asn Tyr Ala Thr Asp Gly Pro

441 450 459 468 477 486  
 ATA CCA TTA CCC AGT AAA GTT TCA AAC CTA ACA GAC TTC TAT CTA ACA ATC TCC  
 Ile Pro Leu Pro Ser Lys Val Ser Asn Leu Thr Asp Phe Tyr Leu Thr Ile Ser

495 504 513 522 531 540  
TAT AAA CTT GAG CCC AAG AAC GGC CTG CCA ATT AAC TTC GCA ATA GAA TCC TGG  
Tyr Lys Leu Glu Pro Lys Asn Gly Leu Pro Ile Asn Phe Ala Ile Glu Ser Trp

549 558 567 576 585 594  
TTA ACG AGA GAA GCT TGG AGA ACA ACA GGA ATT AAC AGC GAT GAG CAA GAA GTA  
Leu Thr Arg Glu Ala Trp Arg Thr Thr Gly Ile Asn Ser Asp Glu Gln Glu Val

603 612 621 630 639 648  
ATG ATA TGG ATT TAC TAT GAC GGA TTA CAA CCG GCT GGC TCC AAA GTT AAG GAG  
Met Ile Trp Ile Tyr Tyr Asp Gly Leu Gln Pro Ala Gly Ser Lys Val Lys Glu

657 666 675 684 693 702  
ATT GTA GTC CCA ATA ATA GTT AAC GGA ACA CCA GTA AAT GCT ACA TTT GAA GTA  
Ile Val Val Pro Ile Ile Val Asn Gly Thr Pro Val Asn Ala Thr Phe Glu Val

711 720 729 738 747 756  
TGG AAG GCA AAC ATT GGT TGG GAG TAT GTT GCA TTT AGA ATA AAG ACC CCA ATC  
Trp Lys Ala Asn Ile Gly Trp Glu Tyr Val Ala Phe Arg Ile Lys Thr Pro Ile

765 774 783 792 801 810  
AAA GAG GGA ACA GTG ACA ATT CCA TAC GGA GCA TTT ATA AGT GTT GCA GCC AAC  
Lys Glu Gly Thr Val Thr Ile Pro Tyr Gly Ala Phe Ile Ser Val Ala Ala Asn

819 828 837 846 855 864  
ATT TCA AGC TTA CCA AAT TAC ACA GAA CTT TAC TTA GAG GAC GTG GAG ATT GGA  
Ile Ser Ser Leu Pro Asn Tyr Thr Glu Leu Tyr Leu Glu Asp Val Glu Ile Gly

873 882 891 900 909 918  
ACT GAG TTT GGA ACG CCA AGC ACT ACC TCC GCC CAC CTA GAG TGG TGG ATC ACA  
Thr Glu Phe Gly Thr Pro Ser Thr Thr Ser Ala His Leu Glu Trp Trp Ile Thr

927 936 945 954  
AAC ATA ACA CTA ACT CCT CTA GAT AGA CCT CTT ATT TCC TAA 3'  
Asn Ile Thr Leu Thr Pro Leu Asp Arg Pro Leu Ile Ser \*

Figure 18b(continued)

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/22623

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : C07H 21/04; C12N 1/20, 1/14, 5/00, 9/38, 9/42; C08B 30/04

US CL : 435/207, 209, 252.3, 254.11, 274, 275, 320.1, 325; 536/23.2

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/207, 209, 252.3, 254.11, 274, 275, 320.1, 325; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	GRABNITZ et al. Structure of the $\beta$ -Glucosidase Gene bglA of Clostridium thermocellum: Sequence Analysis Reveals a Superfamily of Cellulases and $\beta$ -Glycosidases Including Human Lactase/Phlorizin Hydrolase. Eur. J. Biochem. September 1991, Vol. 200, No. 2, pages 301-309, see entire document.	1-3, 5 species II --- 4, 6-11
X --- A	VOORHORST et al. Characterization of the celB Gene Coding for $\beta$ -Glucosidase from the Hyperthermophilic Archaeon Pyrococcus furiosus and Its Expression and Site-Directed Mutation in Escherichia coli. J. Bacteriol. December 1995, Vol. 177, No. 24, pages 7105-7111, see entire document.	1-3, 5 species I and III --- 4, 6-11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

26 MARCH 1998

Date of mailing of the international search report

21 APR 1998

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/22623

**Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:  
1-11, species I-III
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

**B. FIELDS SEARCHED**

Electronic data bases consulted (Name of data base and where practicable terms used):

APS and STN (Bioscience and Patent Indexes): Desulfurococc##, Staphylotherm##, Thermatoga, galactosidase#, glucosidase#, beta galactosidase#, beta glucosidase#. Genbank, EMBL, ESTs1-4, STS, N-Geneseq: Seq. ID Nos.: 1-3 and A-Geneseq, PIR, Swissprot: Seq ID Nos.: 15-17.

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING**

This ISA found multiple inventions as follows:

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. The species are as follows: there are 18 distinct enzymes disclosed in the description, as enumerated in Figs. 1-18 and Table 1.

The claims are deemed to correspond to the species listed above in the following manner: while all the claims form one Group for examination, each of the claims is generic to the 18 enzyme species disclosed.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: each enzyme is a different product, thus has the special technical feature of the recited enzyme, which the other species lack.

